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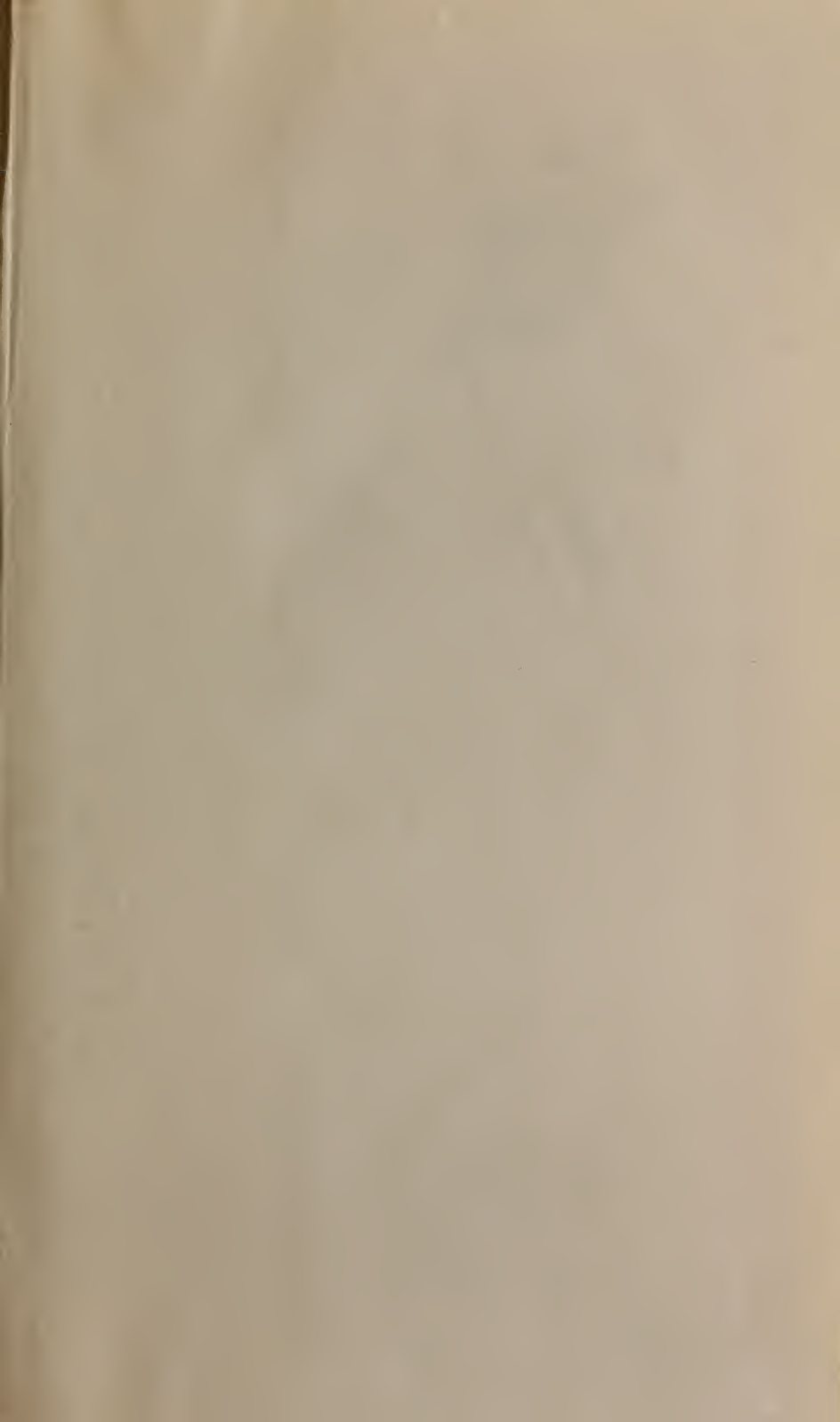
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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF SOILS—BULLETIN No. 36.  
MILTON WHITNEY, Chief.

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FURTHER STUDIES  
ON THE  
PROPERTIES OF UNPRODUCTIVE SOILS.

BY

BURTON EDWARD LIVINGSTON,  
Assisted by CHARLES A. JENSEN, J. F. BREAZEALE,  
F. R. PEMBER, and J. J. SKINNER.



WASHINGTON:  
GOVERNMENT PRINTING OFFICE.  
1907.

## LETTER OF TRANSMITTAL.

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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF SOILS,  
*Washington, D. C., September 29, 1906.*

SIR: I have the honor to transmit herewith the manuscript of a technical paper entitled "Further Studies on the Properties of Unproductive Soils," by Burton Edward Livingston, assisted by Charles A. Jensen, J. F. Breazeale, F. R. Pember, and J. J. Skinner. The results here reported were obtained while Doctor Livingston was in charge of Fertility Investigations, and carry further the line of work forming the subject of Bulletin 28, by the same author, issued in August, 1905. Doctor Livingston severed his connection with this Bureau on January 1, 1906, to accept a position on the staff of the Desert Botanical Laboratory of the Carnegie Institution of Washington.

In accordance with your suggestion this paper has been gone over carefully with Assistant Secretary Hays, who authorizes me to state that he concurs in my recommendation for its publication. I recommend, therefore, that this article be published as Bulletin No. 36 of the Bureau of Soils.

Respectfully,

MILTON WHITNEY,  
*Chief of Bureau.*

HON. JAMES WILSON,  
*Secretary of Agriculture.*

PREFACE.

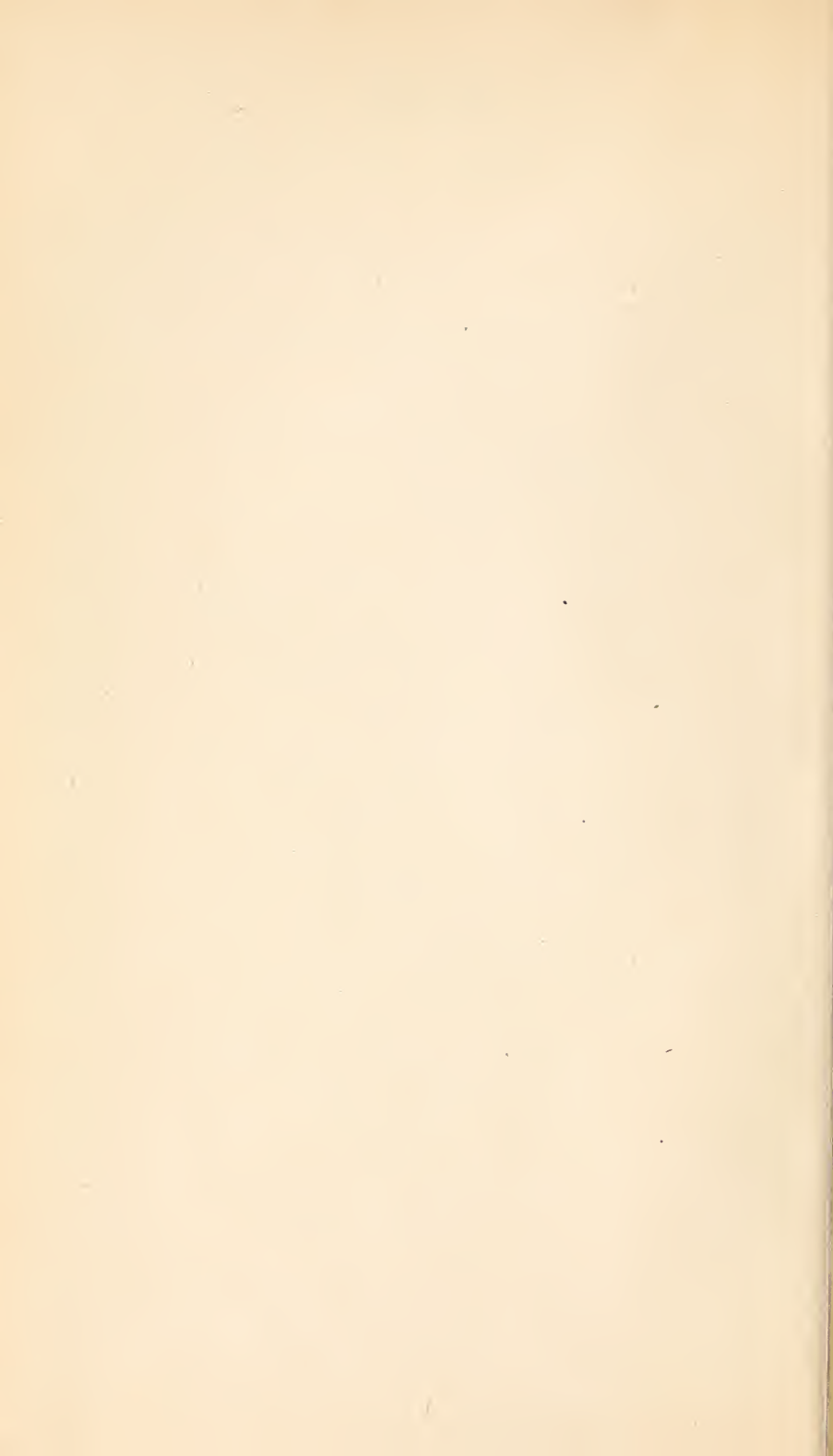
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The investigations which are to be brought forward in the present bulletin follow logically upon those published in Bulletin 28 of this Bureau. In that bulletin evidence was presented which points clearly to the conclusion that the Takoma lawn soil contains substances which are deleterious to plant growth, and that the marked unproductiveness of this soil is due to these substances.

This point having been brought out for one soil, it became essential that other agriculturally poor soils be studied in a similar way. This study has now been made with other soils, and the experimental results in this connection, together with their probable interpretation, will be presented in this bulletin.

That many unproductive soils owe their low agricultural value to the presence of injurious substances is very probable, and stable manure and green manure appear to be, in the majority of cases, of more value in improving such soils than the ordinary chemical fertilizers. The question as to just how these manures exert their beneficial influence assumes therefore an importance quite as great as it has ever possessed when considered in connection with the plant food theory of fertilizer practice. It therefore seemed essential to include some study of this problem in the fertility investigations which are being prosecuted in these laboratories. Enough work has been done in this connection to bring out some new facts which seem to be of the utmost importance in the consideration of the larger problems of agriculture.

In work of the kind here considered, where distilled water takes a very important place as a standard medium for plant growth, it becomes quite essential to know the physiological properties of the water to be used and to obtain if possible a water which is absolutely non-toxic. The ordinary distilled water of the laboratories, condensed in block-tin worms and stored in tin-lined or enamelled receptacles, was found to be quite toxic to wheat plants, and this fact led to an investigation of its toxic properties. The results of these studies indicated that while part of the observed toxicity is due to nonvolatile materials acquired from the storage vessels, the water being much improved by redistillation in glass, yet injurious material remains in the redistilled water and can be removed by absorbing solids. This material must be considered as volatile with the water. An investigation of this matter has been carried out, and since the results are of fundamental importance in reference to the fertility studies which make up the main part of the present publication they will be given at the close of the bulletin.



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# FURTHER STUDIES ON THE PROPERTIES OF UNPRODUCTIVE SOILS.

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## ON THE PRESENCE IN UNPRODUCTIVE SOILS OF SUBSTANCES DELETERIOUS TO PLANT GROWTH.

### LITERATURE.

The idea that agricultural soils may often contain substances which retard plant growth is not a new one, but until quite recently it has taken no prominent part in the modern literature of soil fertility. In De Candolle's *Vegetable Physiology*,<sup>a</sup> published in 1832, is found the most important consideration of this subject among the writings of the earlier authors. The theory of De Candolle was based upon what must now be considered insufficient evidence. This theory was suggested by the idea of von Humboldt and Plenck that the grouping of naturally growing plants into what are now termed plant societies might often be due to materials given off by the roots of the plants inhabiting any area, these materials being injurious to other plants, and therefore keeping the latter out of the area. It also received support from Brugman's<sup>b</sup> statement that *Viola arvensis* excretes substances from its roots, and from the work of Boussingault, who obtained evidence that other plants excrete material in a similar way. De Candolle reasoned that agricultural plants might produce root excretions which were injurious to the plants excreting them. He pointed out an analogy with the case of animals, which may be injured by their own waste products, but which can often thrive on the waste products of other forms. At the suggestion of De Candolle, Macaire<sup>c</sup> made a study of this question and published a review of the literature up to 1831. He carried out a considerable number of original experiments in this connection. Having failed to get evidence of root excretions from seedlings grown in sand and water culture, because, as he thought, the decay of the seed masked the presence of exudations if they occurred, he lifted a number of plants from the open soil, washed their root systems thoroughly, and then

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<sup>a</sup> De Candolle, A. P., *Physiologie végétale*, Paris, 1832.

<sup>b</sup> Brugman, *De mutata humorum in regno organico indole*, 1786.

<sup>c</sup> Macaire-Prinsep, *Memoire pour servir à l'histoire des assolements*; *Mém. de la soc. de physique et l'histoire nat. de Genève*, 5, 282-302 (1832).

placed them in bottles of rain water, which was replaced at intervals of two days. After these cultures had been continued for several changes of water, the presence of exuded materials in the latter was evidenced by a yellow color, smell, taste, and chemical reactions. The chemical reactions by which the exudations were shown were the formation of a precipitate with lead acetate and the clouding or partial coagulation of gelatin solution.

Macaire followed this line of inquiry still further. He observed that when the roots of *Chondrilla muralis* were placed in one vessel of pure water and the stems of the same plants in another similar vessel, exudation was not evident from either roots or stems. He drew from this the conclusion that the exudation which he had observed in ordinary water cultures did not occur, except under normal growing conditions. Plants of *Phaseolus* appeared to excrete more material during the night than during the day.

Macaire was interested in determining whether or not a plant could excrete through its roots a poison which had been absorbed. He placed a portion of the root system of a plant of *Mercurialis annua* in pure water, the remainder of the roots being in a solution of lead acetate. After a few days the lead salt appeared in the vessel which originally contained pure water, a fact which Macaire interpreted as an affirmative answer to the above question.

The same experimenter noted that pease made but a poor growth or died outright in water containing the excretions from the roots of the same plants, and that in order to obtain a normal growth of pease in pure water it was necessary to change the water often. Wheat, on the other hand, thrived well in water charged with the excretions from pea roots.

De Candolle was led by the experiments of Macaire on a considerable number of plant forms to the conclusion that practically all plants give off excretions from their roots, the excretion being, however, much more rapid in some forms than in others. The roots of cereals appeared to be indifferently active in this way, while those of *Chondrilla*, *Sonchus*, *Papaver*, and *Euphorbia* were very active. De Candolle expressed his belief that certain plants excrete from their roots substances which alter the soil in which the roots lie. He cites, as an example of this, *Rhinanthus crista-galli*, which, he says, appears to have an injurious effect upon plants growing contiguous to it.

From these ideas as a starting point, De Candolle formed a theory to explain the well-known fact that continuous cropping of the same plant species often results in decreased growth, while a good growth of every crop may be obtained if rotation is resorted to. He distinguished between true exhaustion of the soil, in which case the soil is to be considered as depleted in respect to the soluble salts necessary for plant growth, and what may be termed false exhaustion, in which

case the cause of the poor crop is to be looked for in the presence of injurious excretions from former crops. De Candolle's theory of crop rotation is, then, that crops, through their root excretions, render the soil unsuitable for the same or closely related plants, but that these root excretions are harmless or even beneficial to distantly related plants. This author expresses his belief that careful experimentation would prove that plants of certain families give off excretions which are very marked in their toxic action and that those of other families are so feebly active in this way that their deleterious effect may be neglected (*Physiologie Végétale*, p. 1499).

Unger <sup>a</sup> and Braconnot <sup>b</sup> pointed out that the phenomena observed by Macaire might have been due to capillary or siphon action of the roots involved. The latter attempted to separate opium from soil in which poppies had been grown, but without success. Walser <sup>c</sup> attempted this in a similar way, also without success.

Boussingault <sup>d</sup> carried out some investigations on this question of root excretions and came to the conclusion that plants do not normally excrete materials from their roots, but may do so in water culture.

Johnson, in "How Crops Grow," page 259, reports that Dr. Alfred Gyde <sup>e</sup> planted a variety of agricultural plants, viz, wheat, barley, oats, rye, beans, peas, vetches, cabbage, mustard, and turnips, in pots filled either with garden soil, sand, moss, or charcoal, and after they had attained considerable growth removed the earth, etc., from their roots by washing with water, using care not to injure or wound them, and then immersed the roots in vessels of pure water. The plants were allowed to remain in these circumstances, their roots being kept in darkness but their foliage exposed to light, from three to seventeen days. In most cases they continued apparently in a good state of health. At the expiration of the time of experiment the water which had been in contact with the roots was evaporated and was found to leave a very minute amount of yellowish or brown matter, a portion of which was of organic and the remainder of mineral origin. Dr. Gyde concluded from his numerous trials that plants do throw off organic and inorganic excretions similar in composition to their sap, but that the quantity is exceedingly small and is not injurious to the plants which furnish them. As pointed out by Johnson, these results are not conclusive proof for nor against root excretions.

<sup>a</sup> Unger, *Einfluss des Bodens auf die Vertheilung der Gewächse*, Vienna, 1836.

<sup>b</sup> Braconnot, H., *Recherches sur l'influence des plantes sur le sol*. *Ann. Chim. Phys.*, 72, 27, 1839.

<sup>c</sup> Walser, *Untersuchungen über die Wurzelausscheidungen*, Tübingen, 1838.

<sup>d</sup> Boussingault, Jean B. J. D., *Rural Economy*, translated by George Law, London, 1845, p. 345.

<sup>e</sup> *Trans. Highland and Agr. Soc.*, 1845-7, p. 273-92.

Since the work of Macaire, as well as that of Brugman, cited by Macaire, is not to be considered as real evidence in the light of modern methods of experimentation, and the theory of De Candolle thus comes to rest, as has already been observed, upon an inadequate foundation of actually observed facts, the foregoing résumé has been given mainly for the sake of historical completeness. But this résumé will also serve to bring out clearly the fact that, to such a pioneer worker in the realm of plant physiology as De Candolle, the idea that infertility is often caused by the presence in the soil of toxic or deleterious substances appeared to be the simplest and most direct means of explaining many of the known facts of plant distribution and of agriculture.

There seems to have been no other serious discussion of this question in the literature until the publication of the work of the Duke of Bedford and Spencer U. Pickering, carried on at the Woburn Experimental Fruit Farm near Bedford, England. The reports of the Woburn Farm for the years 1897 to 1905<sup>a</sup> include accounts of most significant and interesting observations upon the effect of one plant upon another through the apparent intervention of toxic materials. These accounts are worthy of a rather full presentation here.

The two authors just mentioned observed, in their report for 1897, that when the soil surrounding their young apple trees was allowed to be occupied by weeds or was sown to grass, the trees very soon showed a much poorer growth than that exhibited by other trees around which the soil had been kept cultivated. The effect was much more pronounced in the case of grass than in that of weeds. In considering the possible causes of this deleterious effect of the herbage it was pointed out that the grass and weeds probably absorb the nutrient materials of the manure, prevent the normal aeration of the soil, and promote evaporation from the soil both directly through transpiration and indirectly through preventing cultivation and the formation of the usual dust mulch. In this report the authors attributed the bad effects observed mainly to the last-named cause and pointed out that the greater injurious action of the grass was probably due to the fact that it is perennial and active throughout the year, while the weeds dealt with were largely annuals.

Three years later, in the report for 1900, the statement was made that about the worst treatment to which a young apple tree could be subjected was that of sowing the surrounding soil to grass. Trees which were purposely improperly planted and afterwards entirely neglected exhibited a better growth than did trees surrounded by grass. Normally cultivated trees increased in weight in four years from ten to thirty fold, while those surrounded by grass barely

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<sup>a</sup> An account of this work also appeared in a journal article: Pickering, Spencer U., The Effect of Grass on Apple Trees, Jour. Roy. Agr. Soc. Eng., **64**, 365-376 (1903).

doubled their weight. "Neither weeds alone nor weeds coupled with careless planting and total neglect produced such bad effects as the grass."<sup>a</sup> In the yield of fruit the trees surrounded by grass showed a deficiency of 89 per cent, the neglected trees of 82 per cent, and the trees of the weed plots of 55 per cent below the normal. The normally green fruit of the trees surrounded by grass, instead of being green when ripe, was either red or practically colorless and of a waxy aspect and was always undersized. The leaves of these trees were deficient in chlorophyll and were shed about two weeks earlier than those of the normally grown trees.

In this report the authors were inclined to explain the deleterious action of the grass on the ground that it robbed the trees of their supply of nutrient salts and that it diminished the aeration of the roots. The true explanation of the observed phenomena was then sought for by a carefully planned series of experiments, the results of which were presented in the later reports.

In the report of the Woburn Farm for 1903 a number of valuable additional points were brought out. The action of grass was found to be quite as deleterious on old trees as on young trees, and the injury produced was independent of variety or root stock. The roots of trees injured by grass were examined and found to be obviously unhealthy, long and straggling, dark in color, and more slender than normal roots. They showed no tendency to grow downward away from the grass.

Young trees planted in a pasture, with all the sod replaced around them, died during the first season, whereas when even a small area of sod was permanently removed they lived. In another case where holes 3 feet in diameter were opened in the sod, the trees planted, and the sod replaced, at the end of two seasons the trees which were still alive exhibited a growth 32 per cent below the normal, and the mortality of the whole series was 72 per cent.

Trees 8 years old from graft, growing and fruiting normally, were grassed down. Even in the first season the foliage and bark assumed a peculiar light color, characteristic of the trees in other grassed plots, and growth was practically nothing. A small area of sod left around a young tree was shown to retard growth immediately after planting, but as the roots penetrated beyond the grass area an increase in growth accompanied by a more healthy appearance became manifest. Conversely, when the sod was permanently removed for an area of several feet around the trees when first planted, the trees grew well until their roots had penetrated outward to the surrounding grassed soil, after which they began to show the usual and unmistakable effects of grass, the leaves yellowing earlier in autumn than in normal cases and the fruit of green varieties turning red on ripening. When

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<sup>a</sup> Report of Woburn Experimental Fruit Farm, 1900, p. 167.

trees were subjected to the action of grass on one side only the branches on that side showed the grass effects, while the others appeared normally healthy.

The facts just stated argue very strongly against the supposition that the ill effects of the grass are to be considered as due to removal of nutrient salts, to excessive evaporation, or to inadequate aeration. These three possibilities were thoroughly tested by experiments both in the open and in especially constructed iron pots. Using pure sand and natural soil, which had been analyzed to determine its content in nutrient salts, it was definitely shown that the action of grass upon the trees could not be ascribed to its removing nutrient material from the soil. Artificial applications of water showed just as clearly that this effect could not be considered as due to drought conditions induced by the presence of the herbage. Cultures in which air was excluded from the soil, and other similar ones in which the soil was artificially aerated, brought out the fact that exclusion of air could not be the cause of the grass effect. Indeed, during the two first seasons of air exclusion in the field—by means of broad bands of sheet iron and the cementing over of the soil surface—the growth of the trees and yield of fruit were actually in excess of those exhibited by the control trees. Artificial aeration of the soil around trees in grass had no appreciable effect. It seemed possible that the grass might produce its effect through increasing the amount of carbon dioxide in the soil. But determinations of the amount of this gas present in the soil of the various plots showed less carbon dioxide in the grassed-over ground than in that without grass. Furthermore, the artificial introduction of carbon dioxide into the soil of pot cultures had no appreciable injurious effect upon the trees.

The effects of differences in the temperature between the grassed and open soil were also studied, and it was again found that this factor could not possibly explain the injury produced by grass.

Strong root pruning every year stunted the growth of the trees much more than did the presence of grass, but had a different effect. These trees were prevented from dying only by artificial application of water, yet they showed none of the effects produced by grass, their leaves being green and as normal as in well-fed trees.

In view of this mass of evidence the authors concluded in this report, "that this action of grass is not merely a question of starvation in any form, nor of any simple modifications of the ordinary conditions under which a tree can thrive, but that grass has some actively malignant effect on the tree, some action on it akin to that of direct poisoning." Further, "it is no exaggeration to say that if the surface soil were entirely hidden from view, every tree which is grassed over could be identified with the greatest ease; the early bursting of the buds in spring, the green of the leaves in summer, their yellowish hue

in autumn, the color of the bark and the color of the fruit are all equally distinctive. Wherever the grass even approaches a row of trees, then we get some telltale tints, as if the trees were feeling the contagion of their diseased neighbors." In summing up the whole matter, they say, "We can not yet even hazard a suggestion as to whether the poisonous action of the grass is a direct one or only an indirect one, operating through the agency of bacteria; for it is possible that the grass may remove certain bacteria necessary for the growth of the trees, or foster the growth of others prejudicial to tree growth." <sup>a</sup>

In the report for 1905 this question was considered still further. It was pointed out that the presence of grass around the trees did not appear to affect the leaf size, but that the leaf color was a direct indication of the presence of grass. The fruit of the grassed trees, normally green when ripe, or "occasionally tinged with red, were brilliant red in color, with the shaded parts showing a light green, transparent hue; indeed, nothing could be more remarkable than the appearance of these two rows of trees laden with their bright red crops in the midst of 50 or 60 rows of similar trees with their loads of the ordinary green fruits" (p. 47). The authors suggest that the deleterious effect of the grass causes a degeneration of the chlorophyll of the fruit, resulting in the waxy light green or deep red color. They observed that the grassed trees bore quite uniformly a larger crop of fruit than the normal ones and offered the explanation that this is an example of the well-known fact that any check of the growth of a tree, if not too severe, causes an increase in the crop. "In fact, the whole results observed are but an illustration of how a form of treatment, which when carried to excess is highly injurious, may, if adopted in moderation, lead to beneficial results. It is a case of a poison proving to be valuable in minute doses; but the beneficial dose is a very small one in this case" (p. 48).

An investigation was made into the question of what fraction of the entire root system of a tree had to be subjected to the grass treatment in order that the effect should be observed. The grassed area began  $5\frac{1}{2}$  feet from the stem of the trees studied. A trench was subsequently dug along the line separating the grassed from the ungrassed area, and all roots which extended outward beyond this trench were carefully removed. It was found that the air-dry weight of the roots which had penetrated into the grassed soil varied from 0.9 ounce to 2.4 ounces, while the thickest root cut was three-sixteenths inch in diameter in one variety and seven-sixteenths in another. "It will thus be seen that the roots entering the grassed area are almost infinitesimal in amount and can not represent more than one one-thousandth or one two-thousandth part of the whole root system of the trees.

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<sup>a</sup> Report of the Woburn Experiment Fruit Farm, 1903, pp. 48-50.

Yet they must have conveyed something to the trees which has been sufficient to modify the whole character of the crop. This points strongly to the view that the action of the grass is due to some active poison, and it is equally conclusive against the view (if, indeed, further evidence on that point were necessary) that the action can be explained by the grass depriving the trees of the necessary moisture or nourishment" (p. 49).

A number of different varieties of grass tried seemed to have about the same effect. Clover, while stunting the tree growth, failed to produce the pale color of the leaves, probably as the authors point out, on account of the increased amount of nitrates accompanying the clover treatment, which might well overcome the chlorotic action otherwise observed.

The soils on which the work just reviewed was carried out are shallow and underlain by chalk or similar impervious formations, so that the tree roots can not penetrate very deeply below the surface. It may be that the ill effects of grass here observed would not have been manifest had the soil been deeper, and this may explain why it is that, in certain parts of the United States and elsewhere, the growing of grass in apple orchards is not found to be harmful. The main interest lies in the fact that these careful observers and experimenters have been forced to the conclusion that on these soils grass is markedly injurious to fruit trees (observations were made which show the effect of grass on pears, plums, and cherries to be very similar to that so thoroughly studied in the case of apples) and that this injury is undoubtedly due to an active poison resulting, directly or indirectly, from the growth of the grass.

That substances detrimental to plant growth may exist in the soil has been indicated by the work of Woods,<sup>a</sup> who, in a study of the mosaic disease in tobacco, found evidence for believing that this disease may be favored by the action of a substance apparently derived from the decay of tobacco roots in the soil. He concludes that the cause of the mosaic disease is an oxydase and that it can remain without destruction in the soil for some time.

In 1904 Livingston<sup>b</sup> showed that the peculiar characters of bog plants—which had been somewhat indefinitely attributed, by Schimper and others, to the presence of dissolved materials in the soil water—could not be considered as due to too high a concentration, effective through the osmotic pressure of the water. It was found that bog waters from a number of different and widely separated localities exhibited a freezing point scarcely at all above that of water samples taken from neighboring streams and lakes.

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<sup>a</sup> Woods, A. F., Observations on the mosaic disease in tobacco, U. S. Dept. Agr., Bureau of Plant Industry, Bul. 18, 1902. Also see a paper by the same author, Centr. für Bakt., Abt. 2, Band 5, 1899.

<sup>b</sup> Livingston, B. E., Physical Properties of Bog Water, Bot. Gaz., **37**, 383-385 (1904).

The same author, following out the other logical alternative, showed in 1905<sup>a</sup> that bog waters often contain dissolved materials which affect the growth of a small green alga, *Stigeoclonium*, in exactly the same manner as it is affected by mineral poisons.

He ascribes the presence of peculiarly adapted plants in bogs to the occurrence of toxic bodies therein, and regards the peculiar behavior of *Stigeoclonium* in bog water as due to chemical stimulation. Furthermore, he remarks, "The stimulating substances are most markedly present in water from those swamps whose vegetation is most definitely of the bog type. They are absent from river swamps and large lakes. In water from swamps whose vegetation is of a character intermediate between those of the river swamp and the bog, they are present to some degree, their amount being roughly proportional to the extent of the xerophilous character of the vegetation." This author was able to establish the fact that the toxicity of bog water is not at all proportional to its acidity; "while high acidity is always accompanied by the presence of stimulating substances, these substances are not necessarily accompanied by high acidity."

Transeau,<sup>b</sup> working also on the relation of the soil conditions to plant growth in bogs, had previously shown that field sorrel (*Rumex acetosella*) showed peculiar leaf modifications when grown in ordinary dry soil, and that the same modifications were more or less evident when the plants were grown either in wet bog soil at ordinary temperatures or in ordinary soil which was kept moist and much colder than the air. The leaf characters of plants grown in ordinary dry soil were fully reproduced by growing the sorrel in wet bog soil which was also kept cold. It thus appears that while low soil temperature has an influence in bringing about the leaf modifications observed by this author, yet the bog soil itself, aside from its temperature, has a marked influence in the same direction. This may be regarded as evidence in support of the idea advanced by Livingston, that bog soils contain substances which have a poisonous action upon ordinary plants.

This practically completes the literature regarding the presence of toxic substances in soils up to the publication of Bulletin 23, of this Bureau, in which it was shown that the good or bad properties of certain agricultural soils are transmitted to their aqueous extracts, and that differences in the content of these extracts in mineral salts will not at all explain the differences observed in cultures of wheat grown therein. These facts are distinctly in favor of the idea that the poor soils experimented upon contained injurious substances

<sup>a</sup> Livingston, B. E., Physiological Properties of Bog Water, Bot. Gaz., 39, 348-355 (1905).

<sup>b</sup> Transeau, E. N., On the Development of Palisade Tissue and Resinous Deposits in Leaves, Science, n. s., 19, 866-867 (1904).

which were sufficiently soluble in water to be present in the extract in amounts adequate to produce a marked effect upon the growth of wheat plants.

Further and still more convincing evidence in the same direction has recently been brought forward in Bulletin 28, of the Bureau of Soils. In that publication it is shown that the unproductiveness of a very poor soil from Takoma Park, Md., is largely due to the presence of injurious substances which check the growth of plants. It is further indicated by experiments that although this soil is acid, yet its acidity is not the main cause of the poor growth of plants therein. In this regard the aqueous extract of this soil and presumably the natural soil solution agree with the bog waters studied by Livingston. It appears probable that these toxic substances are organic in their nature, and it is possible that they may result, in part at least, from the decay of vegetable matter in the soil. How important a part soil bacteria may play in this connection is still an unsolved problem. Evidence is also presented in Bulletin 28 to the effect that wheat plants do, either directly or indirectly, cause the appearance in the soil or water in which they are grown of substances injurious to wheat itself. Further studies of these phenomena will be embodied in the present bulletin.

Breazeale<sup>a</sup> has recently shown that the growth of wheat seedlings in weak solutions, such as soil extracts, may be improved to a considerable degree by increasing the concentration of the medium through the addition of certain salts thereto. The medium must not, however, be made too concentrated or a deleterious effect will be produced. The same author also showed that the beneficial effect produced by increasing the concentration of a weak nutrient solution appears to be of the same order, whether the salts added are nutrients for the seedlings or not. Thus calcium sulphate and sodium chloride, neither of which could be considered as markedly improving the nutrient value of the solution used (which contained total salts to the amount of 15 parts per million), produced a marked beneficial effect. He calls attention to the fact that absorbing solids produce the same effect upon weak nutrient solutions as does an increase in concentration. From this he argues that since the absorbing solids appear to act through removal of injurious substances given off by the plant roots (see also Bulletin 28), the salts added in his experiments affect the formation or action of such toxic substances by mere physical concentration. Breazeale also showed that these phenomena take place in sand as well as in water culture.

The same author,<sup>b</sup> in a paper on the effects of absorbing solids upon

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<sup>a</sup> Breazeale, J. F., Effect of the Concentration of the Nutrient Solution upon Wheat Cultures, *Science*, n. s., **22**, 146-149 (1905).

<sup>b</sup> Breazeale, J. F., Effect of Certain Solids upon the Growth of Seedlings in Water Cultures, *Bot. Gaz.*, **41**, 54-63 (1906).



WHEAT SEEDLINGS GROWN IN EXTRACT OF DEPARTMENT SOIL WITH STABLE-MANURE TREATMENT.

[1. Extract of Department soil, untreated. 2. The same plus manure extract. 3. The same plus ash of manure extract as sulphates. 4. The same plus ash of manure extract as nitrates.]





FIG. 1.—WHEAT PLANTS GROWN IN EXTRACT OF TAKOMA LAWN SOIL WITH DIFFERENT TREATMENTS OF FERRIC HYDRATE.

- [1. Extract of soil, untreated. 2. Extract of soil plus ferric hydrate used the fifth time. 3. Extract of soil plus ferric hydrate used the first time. 4. Extract of soil, untreated. 5. Extract of soil plus ferric hydrate used the sixth time. 6. Extract of soil plus ferric hydrate used the first time.]



FIG. 2.—WHEAT PLANTS GROWN IN EXTRACT OF DEPARTMENT SOIL WITH STABLE-MANURE TREATMENTS.

- [1. Extract of Department soil, untreated. 2. The same plus manure extract. 3. The same plus ash of manure extract as sulphates. 4. The same plus ash of manure extract, with addition of ammonium nitrate, equivalent to nitrogen of manure extract. 5. The same plus ash of manure extract as nitrates, with addition of ammonium carbonate, equivalent to nitrogen of manure extract. 6. The same plus ammonium nitrate equivalent to nitrogen in manure extract.]



the growth of seedlings, has shown that poor soil extracts are greatly improved for the growth of wheat seedlings by the addition of absorbing solids, these results agreeing, so far as they lie in the same field, with those expressed in Bulletin No. 28. He concludes, "(1) that extracts of certain soils are toxic to wheat seedlings in water culture, and that this toxicity is removed wholly or in part by carbon black, calcium carbonate, ferric hydrate, and other solids; (2) that the toxic substances of ordinary distilled water may be removed by ferric hydrate or carbon black; (3) that the roots of wheat seedlings give off substances which are toxic to themselves and that these substances can be made inactive by the presence of the last-named solids in the culture medium; (4) that the presence of ferric hydrate and carbon black in the solution seemingly accelerates to a marked degree the development of roots, causing them to surpass the tops in growth."

That organic matter deleterious to plant growth is present in the so-called acid soils of the eastern part of the United States has been assumed by practically all experimenters who have dealt with these soils.<sup>a</sup> These workers have found such soils to turn blue litmus paper red, and have interpreted this to denote the presence in the soil of soluble acids. As has recently been pointed out,<sup>b</sup> a soil whose solution is perfectly neutral to litmus may exhibit this reaction through the difference in absorptive power of the soil on the one hand and the paper on the other. The soil, on account of its high absorptive power for bases, removes these from the blue or neutral litmus paper, thus leaving the red dye on the paper. Therefore the litmus paper test can not be considered as throwing any light upon the question as to whether or not the soil is actually acid. The only way to get evidence in this regard is obviously to work with an aqueous soil extract and a very sensitive solution of the indicator. It seems probable, from the evidence of Bulletin No. 28 and that to be presented later, that where the soil is actually acid the acidity is to be regarded rather as an indication of the presence of injurious bodies, which may or may not be themselves acid, than as evidence that free acids are the direct cause of the poor growth of crops.

The well-known observation of gardeners that soil in which potted plants have been grown is unsuited for use in potting other plants or in repotting the same plants, may be considered as an indication that such soil contains injurious material of some sort. The fact that gardeners have adopted the term "sour soil" for this is not to be taken as seriously bearing upon the question as to whether the toxic substances which appear to be present are really effective because of their acid reaction. The term "sour" as here used probably refers

<sup>a</sup> See, especially, H. J. Wheeler's studies carried out at the Rhode Island Agricultural Experiment Station, and published in the bulletins of that station.

<sup>b</sup> Bul. 30, Bureau of Soils, U. S. Dept. of Agr.

to the smell of the soil and to its influence on plants rather than to any actual chemical property.

The voluminous literature which has developed since the time of Liebig on the subject of soil fertility has been almost entirely a consideration of this subject from the standpoint of the "plant food theory" of fertilizers, etc. Since readers of the present bulletin may be assumed to be well acquainted with the general trend of this literature as well as with innumerable specific experiments, and since this literature itself is hopelessly full of contradictions, not only in interpretation but also in experimental evidence, it seems unnecessary to attempt a review of it in this connection.

#### EXPERIMENTATION.

The soils used for these studies were (1) Takoma lawn soil, described in Bulletin 28, of this Bureau; (2) Department soil, a brownish-yellow sandy loam, taken at about 10 feet from the surface on the grounds of the Department of Agriculture at Washington, on the site of the new building now in process of erection; (3) Miami silt loam, from the farm of the Rhode Island Agricultural Experiment Station, at Kingston, R. I.; and (4) Volusia silt loam, from the farm of the Ohio Agricultural Experiment Station, at Wooster, Ohio. The latter soil has been called Wayne silt loam by Selby and Ames, in Bulletin 150 of the Ohio Agricultural Experiment Station.

The Department soil is very unproductive when used in pots, and possesses many of the bad properties of Takoma lawn soil, but to a somewhat less degree. It is, of course, a subsoil and probably exhibits the characteristics of many subsoils of the Atlantic coast region, which are well known to be generally very poor media for plant growth. The properties of Volusia silt loam at Wooster, Ohio, are discussed at length in Bulletin 167 of the Ohio Agricultural Experiment Station, which reports a mass of experimental data brought out by cooperative work between that station and the Bureau of Soils, and from which the evidence here presented in regard to this soil has been taken. The "exhausted" Miami silt loam from Kingston, R. I., is described and its properties discussed in the Eighteenth Annual Report of the Rhode Island Agricultural Experiment Station, pp. 286-323. From this paper are taken the experiments which are presented here regarding this soil. Some of the data to be considered here, concerning Takoma lawn soil, have already been published in Bulletin 28 of this Bureau, and are assembled here in order to present the exact status of these fertility investigations at the present time.

The seeds of the Russian variety of wheat known as "Chul" were germinated in distilled water and the seedlings used for these cultures. The germination took place on a paraffined disk of galvanized iron wire netting of one-fourth inch mesh which was floated upon corks

fixed to its edges so that it was held just at the surface of distilled water in a "granite iron" pan. The water was about 8 centimeters deep, and was changed daily or several times a day in warm weather. When the seedlings had attained a height of about 4 centimeters they were placed in wide-mouthed culture bottles, capacity about 240 c. c., being fixed in place by inserting their stems in small V-shaped vertical grooves cut in the margin of flat cork stoppers. Each seedling was held in position by replacing the cork wedge, which had been removed in cutting the groove, the wedge having been truncated by the removal of sufficient cork along its apical edge to allow space for the stem. A rubber band encircled the stopper above the neck of the bottle to prevent the slipping of the wedges when the stopper was removed.<sup>a</sup> Two bottle cultures were used for each test, ten seedlings being used in each bottle, and the solution was replaced every three or four days during the experiment, which lasted usually from ten to fifteen days. The amount of water lost by transpiration for each culture was determined at each change of the solution, and the sum of these partial losses gives the total transpiration of the culture during the whole period of the experiment. This amount of total transpiration, being proportional to the leaf area and approximately proportional to the green and dry weights of the plant tops,<sup>b</sup> was taken as the main criterion for comparison of growth in the various cultures of any experiment. At the end of the experiment the green and dry weights were also taken in most cases to serve as additional evidence. Where figures are given they represent ten plants from each treatment. It was usually the practice to use ten plants for the photograph and obtain weights on the remaining ten.

The soil extracts were prepared according to the method described in *Bulletins* Nos. 23 and 28 of this Bureau.

The experimental evidence will be considered under the three headings, Existence, Nature, and Origin of the toxic substances. Under the first of these headings the experiments will be taken up in several groups, according to their nature. Evidence will be presented from several different soils.

#### EXISTENCE OF TOXIC SUBSTANCES IN POOR SOILS.

The relative growth of a number of plant cultures in different solutions held in exactly similar containers and standing side by side under exactly similar conditions must be determined by the nature of the solutes contained in the culture medium. It is thus clear that

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<sup>a</sup> For a description of this method the reader is referred to *Bul.* 28, Bureau of Soils, U. S. Dept. of Agr., and also to Livingston, B. E., "A Simple Method for Experiments with Water Cultures, *Plant World*, 9, 13-16 (1906).

<sup>b</sup> Livingston, B. E., Relation of Transpiration to Growth in Wheat, *Bot. Gaz.*, 40, 178-195 (1905).

if, under these conditions, pure distilled water produces a better growth than does the soil extract there must be some harmful material contained in the latter. The source of this harmful material must logically be the soil which furnished the extract. One of the most convincing lines of evidence which has been thrust upon us in the progress of these fertility experiments is the fact that the above supposition is actually true in the case of the most unproductive soils experimented upon.

Such a result has already been reported in Bulletin No. 28 of this Bureau, where it was shown that plants grown in pure carbon-treated distilled water gave a transpiration of 151 as against 100 for the plants grown in the extract of the Takoma lawn soil. Another experiment with Department soil extract gave a transpiration of 123 in redistilled water as against the 100 for the extract culture. A mixture of equal parts of the extract and redistilled water gave a transpiration of 105.

A result similar to the one shown by the preceding experiments was obtained by this Bureau, working in cooperation with the Rhode Island Agricultural Experiment Station, upon extract of exhausted Miami silt loam from the farm of that station, at Kingston, R. I. In this experiment filtered rain water was used in place of redistilled or carbon-treated water. The plants in the filtered rain water gave a transpiration of 109, and a green weight of the tops of 100, compared with 100 for both transpiration and green weight of the tops of the plants grown in the soil extract.

While the gain in plant growth in favor of the rain water is slight, according to transpiration, and while no gain is shown by green weight, it is apparent that the extract must have contained injurious material; the only difference shown is in favor of the water. It must be remembered also that the soil extract contained a much larger amount of nutrient salts than did the water, and that if the presence of these salts tended to increase plant growth, as it undoubtedly does to some extent, and as is supposed by the prevalent theory of soil fertility, the slight superiority by transpiration, and the equality by green weight, of the plants in rain water, must be related to some tendency of the extract to retard growth and thus neutralize the accelerating effect of the nutrient salts derived from the soil.

Another experiment in the same line involved a comparison of carbon-treated distilled water (which, as stated later, we believe to be the least toxic water thus far obtained) with an extract of the last-named soil prepared with carbon-treated water and diluted to twice its original volume with the same water, in which the plants grown in the mixture of water and soil extract gave a transpiration of 107 and green weight of tops of 135, as compared with 100 for the plants grown in the extract undiluted.

Cultures were made comparing a nutrient solution of 170 parts per million of total salts, prepared from carbon-treated distilled water, with a nutrient solution made up in exactly the same way, but with extract of exhausted Miami silt loam in place of pure water, the soil extract being prepared from carbon-treated distilled water. In the latter treatment the soil was allowed to act upon the water before it was used to make up the nutrient solution.<sup>a</sup> The plants grown in the nutrient solution made up with the soil extract gave a transpiration of 79 and green weight of the tops of 88, as compared with 100 for the plants grown in the normal nutrient solution.

From this experiment it is seen that, judged by both criteria, the soil extract has injured rather than benefited the solution. It can not be assumed in this experiment that the nutrient solution here used was so concentrated that the slight increase in solutes obtained by the use of soil extract instead of pure water was attended by retardation of growth due to too high a concentration, for the optimum strength for the growth of wheat seedlings in this solution was found to be about 300 parts per million and the soil extract contained only about 68 parts per million of total salts. Thus the solution which gave the lesser growth contained about 238 parts per million of salts, a concentration which falls well below the optimum for the seedlings used. These results point toward the same conclusions as those reached in the preceding experiments, namely, that the soil contains water-soluble materials which retard the growth of wheat.

Another method of attacking this same question is to make a quantity of soil extract with nontoxic water and then distill a portion of it in glass, comparing the distillate with the original extract. This was done with the extract of Takoma lawn soil. The portion subjected to distillation was of course not completely distilled over; it was boiled in the retort till about one-half had been distilled off, and the plants grown in the distillate gave a transpiration of 257, as against 100 for the plants grown in the untreated soil extract.

From this it is seen that distilling the extract of this soil produced an improvement in the growth of the plants of 157 per cent. We are thus confronted with the fact that glass-distilled water, together with whatever volatile material may have been in the soil extract, produced a much better growth than did the extract itself, the latter containing considerable amounts of nutrient salts. The only possible explanation of this is that the soil extract contained toxic substances which not only counteracted any tendency toward a beneficial effect due to the presence of nutrient salts, but, in addition, actually retarded the growth of the seedlings to a marked degree.

As will be brought out in the sequel, finely divided, insoluble solids have the power of absorbing and thus removing from solution cer-

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<sup>a</sup>This experiment has been described in Bul. 28, Bureau of Soils, U. S. Dept. of Agr.

tain classes of dissolved substances, including the toxic substances of ordinary distilled water, copper sulphate, etc. It has been shown in Bulletin 28 of this Bureau that the toxic substances contained in the extract of Takoma soil are likewise largely removed by the same treatment. That the effect of the solids can not be attributed to any hypothetical stimulation of a mechanical nature is clearly proved by the fact that when the solution is shaken with the solid and the latter is then entirely removed a very marked improvement in growth conditions is the result.

*Experiment I.*—In Experiment I the soil extract was treated with the solid by shaking and then filtering. This was performed upon extract of Department soil. The relative data for transpiration and dry weight of tops are given in Table I. The use of the term "Relative Transpiration" in this and the following tables and in the discussions of this bulletin simply denotes the relation of the different amounts of water loss to the standard culture of the particular series involved, and has no relation whatever to the more technically physiological term, "Relative Transpiration," as introduced by Livingston (1906) in Publication No. 50 of the Carnegie Institution.

TABLE I.—*Comparison of extract of Department soil with the same extract treated with carbon, with ferric hydrate, and with aluminum hydrate.*

| Treatment.                               | Relative transpiration. | Relative dry weight of tops. |
|------------------------------------------|-------------------------|------------------------------|
| Extract of Department soil.....          | 100                     | 100                          |
| Same, treated with carbon.....           | 183                     | 100                          |
| Same, treated with ferric hydrate.....   | 247                     | 120                          |
| Same, treated with aluminum hydrate..... | 206                     | 121                          |

From Table I it appears that the action of the insoluble absorbing solids has increased the productiveness of this soil extract to an extent amounting to from 83 to 147 per cent transpiration and from nothing to 21 per cent dry weight of tops. The failure of carbon to produce a marked effect upon dry weight is exceptional; in most cases the dry weights follow about the same order as do the transpiration figures. Ferric hydrate is almost always somewhat more beneficial upon this soil extract than carbon black, while the effectiveness of aluminum hydrate lies between that of the other two solids.

*Experiment II.*—Another experiment upon the "exhausted" Miami silt loam at Kingston, R. I., is here given. The experiment consisted of cultures in soil extract and in the same extract treated with the following substances: Ammonia-free ferric hydrate (made from sodium hydrate and ferric chloride); the same ferric hydrate after it had been treated with ammonia and then thoroughly washed; ferric hydrate made from ferric nitrate and ammonia, and chemically

pure metallic iron. The ferric hydrates were shaken with the soil extract and then filtered out; the iron was allowed to remain in the extract during the growth of the plants.

The relative results, according to transpiration and green weight, are given in Table II.

TABLE II.—*Comparison of extract of exhausted Miami silt loam with the same extract treated with three forms of ferric hydrate and with the addition of metallic iron.*

| Treatment.                                                           | Relative transpiration. | Relative green weight of tops. |
|----------------------------------------------------------------------|-------------------------|--------------------------------|
| Extract of Miami silt loam.....                                      | 100                     | 100                            |
| Same, treated with ammonia-free ferric hydrate.....                  | 283                     | 100                            |
| Same, treated with ammonia-treated ferric hydrate.....               | 167                     | 103                            |
| Same, treated with ferric hydrate made from nitrate and ammonia..... | 240                     | 105                            |
| Same, with addition of metallic iron.....                            | 111                     | 98                             |

The different forms of ferric hydrate are seen to have all had a marked accelerating influence on the transpiration, while the last two of them have affected green weight in the same manner but only to a slight degree. Metallic iron was included in the experiment in order to determine whether the effect of ferric hydrate upon this soil extract could be considered as in any degree due to iron itself. The experiment answers this question clearly in the negative, this fact strengthening the argument already given, from the insoluble nature of the ferric hydrate, that this solid can not have its effect otherwise than through its absorbent action.

Another test was carried out with extract of "exhausted" Miami silt loam and the same extract treated with carbon black. Transpiration was obtained for the last eight days of the experiment only, and the plants in the carbon-treated soil extract transpired 318 and gave a green weight of 153 as against 100 for the plants in the untreated extract. It therefore becomes clear that carbon black, as well as ferric hydrate, is markedly beneficial when applied to extract of this soil. Aluminum hydrate was not tested in this connection.

With Volusia silt loam, at Wooster, Ohio, were obtained similar, though less marked, results from treatment of the extract with carbon black. The plants grown in the carbon-treated extract gave a transpiration of 108 and a green weight of 103, as compared with 100 for the plants grown in the untreated extract.

An experiment performed upon extract of Takoma lawn soil treated with ferric hydrate has been given in Bulletin 28, but is repeated here for completeness. In this experiment the treatment resulted in an increase of transpiration amounting to 66 per cent for the treated extract. Another similar experiment showed an increase of 31 per cent due to the same treatment. An experiment similar to

the last was carried out, using carbon black instead of ferric hydrate, and a gain of 26 per cent in transpiration was produced by the treatment. In a similar experiment a gain of 5 per cent was exhibited.

When the absorbing solid is not filtered out of the soil extract, but is allowed to remain in the culture bottle during the growth of the plants, it has as great, and sometimes even greater, effect than when it is filtered out. Several experiments in which the absorbing solid was not filtered out will now be reviewed.

*Experiment III.*—Under this head will be grouped a series of experiments upon extract of Takoma lawn soil with addition of ferric hydrate. These have been described more fully in Bulletin 28. The duration of the separate tests, the number of plants used for each treatment, and the relative results, according to the criterion of transpiration, are given in Table III.

TABLE III.—*Comparison of extract of Takoma lawn soil with the same extract plus ferric hydrate.*

[The transpiration from the culture in untreated soil extract is uniformly considered as 100.]

| Duration of experiment.         | Number of plants for each treatment. | Relative transpiration. |
|---------------------------------|--------------------------------------|-------------------------|
| June 17 to June 25, 1904.....   | 24                                   | 201                     |
| June 18 to June 25, 1904.....   | 24                                   | 212                     |
| March 20 to April 4, 1905.....  | 36                                   | 205                     |
| March 28 to April 17, 1905..... | 36                                   | 174                     |
| March 31 to April 18, 1905..... | 36                                   | 141                     |

*Experiment IV.*—Under this head will be presented a series similar to the last, also described in Bulletin 28, but in which carbon black was used in place of ferric hydrate. Table IV presents the relative results and is similar to Table III. Thirty-six plants were used for each treatment throughout.

TABLE IV.—*Comparison of extract of Takoma lawn soil with the same extract plus carbon black.*

[The transpiration from the culture in untreated soil extract is uniformly considered as 100.]

| Duration of experiment.            | Relative transpiration. |
|------------------------------------|-------------------------|
| October 3 to October 17, 1904..... | 113                     |
| March 20 to April 4, 1905.....     | 157                     |
| March 28 to April 17, 1905.....    | 148                     |
| March 31 to April 18, 1905.....    | 130                     |

An experiment to test the productiveness of extract of Department soil with and without ferric hydrate was carried out, in which the plants grown in the extract plus the solid gave transpiration of 188 and green weight of 111, compared with 100 for the plants grown in the untreated extract. Carbon black has a similar effect.

*Experiment V.*—This is a test of the effect of addition of carbon black and ferric hydrate to extract of Volusia silt loam from Wooster, Ohio. The relative variations in transpiration and in green weight of the entire plants are given in Table V.

TABLE V.—*Comparison of extract of Volusia silt loam with the same extract with addition of carbon black and of ferric hydrate.*

| Treatment.                        | Relative transpiration. | Relative green weight of entire plants. |
|-----------------------------------|-------------------------|-----------------------------------------|
| Extract of Volusia silt loam..... | 100                     | 100                                     |
| Same, + carbon black.....         | 119                     | 102                                     |
| Same, + ferric hydrate.....       | 129                     | 113                                     |

*Experiment VI.*—The effect of adding to extract of Volusia silt loam a mixture of carbon black and ferric hydrate was also determined in several tests. The relative results from two of these experiments are given in Table VI.

TABLE VI.—*Comparison of extract of Volusia silt loam with the same extract with addition of a mixture of carbon black and ferric hydrate.*

[The data for the untreated extract are considered as 100.]

| Duration of experiment.       | Number of plants for each treatment. | Relative transpiration. | Relative green weight of tops. |
|-------------------------------|--------------------------------------|-------------------------|--------------------------------|
| April 27 to May 11, 1905..... | 36                                   | 136                     | 115                            |
| May 15 to May 23, 1905.....   | 36                                   | 132                     | 158                            |

*Experiment VII.*—A test of the effect produced by addition of carbon black and of ferric hydrate to extract of "exhausted" Miami silt loam from Kingston, R. I., gave the relative data which are given in Table VII. Transpiration was obtained for the last eight days only.

TABLE VII.—*Comparison of extract of "exhausted" Miami silt loam with the same extract with addition of carbon black and of ferric hydrate.*

| Treatment.                      | Relative transpiration. | Relative green weight of tops. |
|---------------------------------|-------------------------|--------------------------------|
| Extract of Miami silt loam..... | 100                     | 100                            |
| Same, + carbon black.....       | 259                     | 168                            |
| Same, + ferric hydrate.....     | 204                     | 161                            |

Another test of the effect of addition of ferric hydrate to this soil extract showed a gain of 247.6 per cent by transpiration and 114 per cent by green weight, considerably more than that shown in Table VII. A test of the effectiveness of addition of carbon black showed

a gain of 177.5 per cent by transpiration and 45 per cent by green weight, less than that shown in Table VII.

*Experiment VIII.*—The evidence so far given in regard to the action of the absorbing solids seems to indicate that the improvement in plant growth which follows their use is due to their power to remove from the soil extract substances deleterious to wheat seedlings. If this be the true explanation of the action of these solids, their repeated use should decrease their effect. The absorbing power of a given mass of solid must be supposed to be limited, and when it has absorbed material until this limit is approached its ability to absorb more should be markedly decreased. This was tested by growing cultures of wheat seedlings in extract of Takoma soil with addition of ferric hydrate and leaving the original mass of solid in the bottles at the time the solutions were changed, repeating this process through a number of successive wheat cultures. This experiment was begun May 4 and continued until September 6, 1905, seven different series of cultures being grown in succession with the same mass of absorbing solid. In every experiment untreated soil extract was used for comparison, and the percentage gain or loss in growth by transpiration and by green weight and dry weight of tops was determined. The data are given in Table VIII. The appearance of the plants in these cultures followed the transpiration figures.

TABLE VIII.—*Effect of repeated use of ferric hydrate in wheat cultures in extract of Takoma lawn soil.*

| Number of series. | Date of series.                     | Per cent variation. |                       |                     |
|-------------------|-------------------------------------|---------------------|-----------------------|---------------------|
|                   |                                     | Transpiration.      | Green weight of tops. | Dry weight of tops. |
| 1                 | May 4 to May 19, 1905.....          | +181                | + 9                   | +18                 |
| 2                 | May 20 to June 6, 1905.....         | +116                | +13                   | 0                   |
| 3                 | June 6 to June 17, 1905.....        | + 4                 | +16                   | +12                 |
| 4                 | June 19 to July 7, 1905.....        | + 63                | .....                 | .....               |
| 5                 | July 21 to August 2, 1905.....      | + 10                | + 8                   | +15                 |
| 6                 | August 3 to August 21, 1905.....    | - 17                | .....                 | .....               |
| 7                 | August 20 to September 6, 1905..... | - 12                | .....                 | -45                 |

From these results it is apparent that the efficiency of ferric hydrate added to extract of this soil decreases with repeated use of the solid, and that the solid at length becomes injurious instead of beneficial.

*Experiment IX.*—In each of the last three series of the experiment from which the data just given were derived a treatment of the soil extract with unused ferric hydrate was inserted, so as to give evidence of the comparative effectiveness of the used and the unused solid when the cultures were grown under exactly the same external conditions of weather, etc. In series 5 and 6 the amount of fresh ferric hydrate used was approximately the same as that of the solid repeatedly used,

but in the last series (7) the amount of the fresh hydrate was made considerably less than that of the used hydrate, so as to bring out clearly whether the difference in effects observed could possibly be due to a difference in the amount rather than in the quality of the ferric hydrate. The data from these tests are given in Table IX.

TABLE IX.—*Effectiveness upon extract of Takoma lawn soil of ferric hydrate used for the first time compared with that of the ferric hydrate used for the fifth, sixth, and seventh times, respectively.*

| Number of series. | Date of series.                     | Per cent variation. |       |               |       |             |       |
|-------------------|-------------------------------------|---------------------|-------|---------------|-------|-------------|-------|
|                   |                                     | Transpiration.      |       | Green weight. |       | Dry weight. |       |
|                   |                                     | Fresh.              | Used. | Fresh.        | Used. | Fresh.      | Used. |
| 5                 | July 21 to August 2, 1905.....      | +319                | +10   | +10           | +8    | +25         | +15   |
| 6                 | August 3 to August 21, 1905.....    | +52                 | -17   | .....         | ..... | .....       | ..... |
| 7                 | August 20 to September 6, 1905..... | +14                 | -12   | .....         | ..... | +58         | -45   |

This table shows clearly that with repeated use in this soil extract the ferric hydrate loses its power to improve the extract for the growth of wheat seedlings, and that it at length has even a retarding effect upon their growth. In spite of the fact that in series 7 the used hydrate was added to the soil extract in somewhat larger quantities than the fresh, there was nevertheless an effect shown amounting to +14 for the fresh and -12 for the used hydrate in transpiration, and +58 and -45 in dry weight of crop.

It thus appears that ferric hydrate has a beneficial effect upon extract of a poor soil, because it absorbs and removes from solution certain injurious substances transmitted from the soil to its extract. It further appears that the solid can continue to absorb these substances only until a certain limit is reached, after which, being thoroughly impregnated, as it were, it ceases to absorb, and hence also ceases to have any improving effect upon the extract. The solid impregnated with toxic materials may even be markedly injurious to the extract in which it is placed, as shown by the results. This might be explained by the supposition that the solid has absorbed to its limit, and then, upon being placed in a somewhat different solution, gives up some of the absorbed material until equilibrium is again established.

If it is true that carbon black and ferric hydrate are beneficial to these soil extracts because the latter contain injurious substances which the solids are capable of absorbing, then these solids should have a much more marked effect upon extract of poor soils than upon those of good soils.

*Experiment X.*—The question just pointed out was tested in the case of the Miami silt loam at Kingston, R. I. The effects of carbon black and ferric hydrate, both when merely shaken with the extract

and when allowed to remain during the growth of the cultures, were determined upon extract of "exhausted" Miami silt loam and upon extract of the same soil when in fair condition resulting from proper care and fertilizer treatment. The data obtained are given in Table X.

TABLE X.—*Comparison of the effects produced by carbon black and ferric hydrate when used upon "exhausted" Miami silt loam with those produced by the same treatments upon Miami silt loam in fair agricultural condition from plats 11 and 29 of the Rhode Island Agricultural Experiment Station.*

| Treatment.                             | "Exhausted" soil.       |                        | Plat 29.                |                        | Plat 11.                |                        |
|----------------------------------------|-------------------------|------------------------|-------------------------|------------------------|-------------------------|------------------------|
|                                        | Relative transpiration. | Relative green weight. | Relative transpiration. | Relative green weight. | Relative transpiration. | Relative green weight. |
| Soil extract.....                      | 100                     | 100                    | 100                     | 100                    | 100                     | 100                    |
| Same, treated with carbon black.....   | 318                     | 153                    | 117                     | 90                     | 146                     | 109                    |
| Same, + carbon black.....              | 259                     | 168                    | 121                     | 69                     | 206                     | .....                  |
| Same, treated with ferric hydrate..... | 240                     | 105                    | 191                     | 98                     | 151                     | 108                    |
| Same, + ferric hydrate.....            | 204                     | 161                    | 185                     | 97                     | 143                     | 102                    |

In every case excepting that of the green weight for the ferric hydrate treatment of soil extract from plat 11 the figures from the extracts of soil from plats 11 and 29 are much lower than those from the "exhausted" soil. This substantiates very well the supposition made above, that the beneficial effects of carbon black and ferric hydrate upon soil extracts are more marked in those from the poorer soils.

The extracts of these unproductive soils are greatly improved for the growth of wheat seedlings by the addition of certain chemicals. Some of these chemicals are plant nutrients, while others are not. The effect of the former class of substances may logically be ascribed to a mere increase in the nutrient material, but that of the latter class must be ascribed to some other cause. The experimental data in this connection will be presented for the different soils separately, and will be followed by a general discussion of the phenomena involved, which will include some additional data of experiments seeming to throw light on certain points of the discussion.

Addition to the extract of Takoma lawn soil of a mixture of calcium sulphate, magnesium acid phosphate, potassium carbonate, sodium nitrate, and ammonium chloride, the salts in chemically equivalent amounts, is followed by a considerable increase in the growth of wheat seedlings. Experiment XI brings out this fact.

*Experiment XI.*—In this experiment the mixture of salts was added in quantities ranging from 1 to 50 parts per million. The relations of the resulting transpiration figures to those given by plants grown in untreated soil extract are given in Table XI.

TABLE XI.—*Effect of mixture of nutrient salts upon extract of Takoma lawn soil.*

[P. p. m.=parts per million.]

| Treatment.                     | Relative transpiration. |
|--------------------------------|-------------------------|
| Soil extract untreated.....    | 100                     |
| Same, + 1 p. p. m. salts.....  | 141                     |
| Same, + 5 p. p. m. salts.....  | 162                     |
| Same, + 10 p. p. m. salts..... | 112                     |
| Same, + 20 p. p. m. salts..... | 141                     |
| Same, + 50 p. p. m. salts..... | 202                     |

The addition of sodium nitrate alone had a similar effect, 60 parts per million added to the untreated soil extract increasing transpiration 39 per cent. Among the nonnutrients which increase the growth of wheat seedlings in extract of Takoma lawn soil, pyrogallol is the most noteworthy. As has been pointed out in Bulletin 28, addition of this substance to the soil extract produced very marked effects. The average increase in growth, for three cultures of 12 plants each, judged by the amount of transpiration, brought about by the addition of 10 parts per million of this substance was shown to amount to 38.6 per cent in an experiment presented in the bulletin just mentioned. In that bulletin it was shown that while larger amounts of the chemical could not be used alone with any great effect yet if the pyrogallol was added to the extract and then the resulting acidity neutralized with trimethylamine an increase of from 47 to 67 per cent could be obtained. In such an experiment 40 parts per million of pyrogallol, neutralized with trimethylamine, was added to the extract of the same soil and gave a transpiration of 135, and green weight of tops of 108, as compared with 100 for the untreated extract.

*Experiment XII.*—In this case pyrogallol was added to the extract and half of the treated solution was immediately neutralized with trimethylamine, the other half remaining unneutralized until the following day, when it was likewise treated with trimethylamine. The chemical was added in amounts of 10 and 40 parts per million.

TABLE XII.—*Effect of pyrogallol neutralized with trimethylamine upon extract of Takoma lawn soil.*

[P. p. m.=parts per million.]

| Treatment.                                                      | Relative transpiration. |
|-----------------------------------------------------------------|-------------------------|
| Soil extract untreated.....                                     | 100                     |
| Same, + 10 p. p. m. pyrogallol, immediately neutralized.....    | 123                     |
| Same, + 10 p. p. m. pyrogallol, neutralized after 15 hours..... | 93                      |
| Same, + 40 p. p. m. pyrogallol, immediately neutralized.....    | 161                     |
| Same, + 40 p. p. m. pyrogallol, neutralized after 15 hours..... | 145                     |

From this experiment it appears that the use of pyrogallol gives the most marked effects when it is immediately neutralized.

*Experiment XIII.*—In this experiment the effect of sodium nitrate on the extract of Department soil was tried. The relative results, according to transpiration, green weight, and dry weight of tops, are given in Table XIII. They show that the effect here is very similar to that upon Takoma soil.

TABLE XIII.—*Effect of sodium nitrate upon extract of Department soil.*

[P. p. m.=parts per million.]

| Treatment.                                   | Relative transpiration. | Relative green weight. | Relative dry weight. |
|----------------------------------------------|-------------------------|------------------------|----------------------|
| Extract of Department soil.....              | 100                     | 100                    | 100                  |
| Same, + NaNO <sub>3</sub> , 50 p. p. m.....  | 142                     | 128                    | 106                  |
| Same, + NaNO <sub>3</sub> , 100 p. p. m..... | 138                     | 147                    | 125                  |
| Same, + NaNO <sub>3</sub> , 200 p. p. m..... | 133                     | 153                    | 119                  |
| Same, + NaNO <sub>3</sub> , 400 p. p. m..... | 155                     | 151                    | 113                  |

Pyrogallol has not been tested in the case of this soil extract, but hydroquinone, a closely related chemical, was found to have a marked effect. Experiment XIV shows such a treatment.

*Experiment XIV.*—The chemical was added to the extract in quantities varying from 2 to 16 parts per million. The results, according to transpiration and dry weight of tops, are given in Table XIV.

TABLE XIV.—*Effect of hydroquinone upon extract of Department soil.*

[P. p. m.=parts per million.]

| Treatment.                            | Relative transpiration. | Relative dry weight. |
|---------------------------------------|-------------------------|----------------------|
| Extract of Department soil.....       | 100                     | 100                  |
| Same, + hydroquinone, 2 p. p. m.....  | 161                     | 126                  |
| Same, + hydroquinone, 4 p. p. m.....  | 174                     | 115                  |
| Same, + hydroquinone, 6 p. p. m.....  | 72(?)                   | 124                  |
| Same, + hydroquinone, 8 p. p. m.....  | 137                     | 132                  |
| Same, + hydroquinone, 12 p. p. m..... | 128                     | 101                  |
| Same, + hydroquinone, 16 p. p. m..... | 95                      | 118                  |

Concentrations of 2 or 4 parts per million appear to be most beneficial in this case. The erratic behavior of the relative transpiration in the case of the third treatment of this series can not be explained. It may be an experimental error. From the data at hand the larger amounts of hydroquinone seem to be not as beneficial or are negative in their effect.

*Experiment XV.*—In this experiment the commonly used fertilizer salts were added to the extract of Volusia silt loam, each at the rate of 50 parts per million. The comparative growths of the different cultures, according to transpiration and green weight, are given in Table XV. From this series it appears that this extract is benefited quite markedly by the addition of all of these salts. Potassium sulphate produced the least effect and potassium nitrate the greatest.

TABLE XV.—*Effect of fertilizer salts on extract of Volusia silt loam.*

| Treatment.                                   | Relative transpiration. | Relative green weight of tops. |
|----------------------------------------------|-------------------------|--------------------------------|
| Extract of Volusia silt loam, untreated..... | 100                     | 100                            |
| Same, + sodium nitrate.....                  | 132                     | 132                            |
| Same, + calcium acid phosphate.....          | 114                     | 108                            |
| Same, + potassium sulphate.....              | 110                     | 129                            |
| Same, + potassium nitrate.....               | 136                     | 157                            |
| Same, + potassium acid phosphate.....        | 134                     | 135                            |

*Experiment XVI.*—A test of the effect of pyrogallol neutralized with trimethylamine upon this soil extract showed a beneficial effect, though this was not as large as was obtained with Takoma lawn soil. The relative results of this experiment are presented in Table XVI.

TABLE XVI.—*Effect of pyrogallol upon extract of Volusia silt loam.*

[P. p. m.=parts per million.]

| Treatment.                                                            | Relative transpiration. | Relative green weight. |
|-----------------------------------------------------------------------|-------------------------|------------------------|
| Extract of Volusia silt loam.....                                     | 100                     | 100                    |
| Same, + pyrogallol, 20 p. p. m., neutralized with trimethylamine..... | 104                     | 110                    |
| Same, + pyrogallol, 40 p. p. m., neutralized with trimethylamine..... | 118                     | 105                    |
| Same, + pyrogallol, 60 p. p. m., neutralized with trimethylamine..... | 121                     | 100                    |

*Experiment XVII.*—In this test the ordinary fertilizer salts were added to extract of “exhausted” Miami silt loam. Transpiration data were taken for the last eight days. These, reduced to a basis of 100 for the control culture, together with the relative green weights, are given in Table XVII. All of the treatments are seen to be markedly beneficial, some of them exceedingly so.

TABLE XVII.—*Effect of fertilizer salts on extract of “exhausted” Miami silt loam.*

[P. p. m.=parts per million.]

| Treatment.                                                                                  | Relative transpiration. | Relative green weight of tops. |
|---------------------------------------------------------------------------------------------|-------------------------|--------------------------------|
| Extract of exhausted Miami silt loam, untreated.....                                        | 100                     | 100                            |
| Same, + $K_2SO_4$ (K 50 p. p. m.).....                                                      | 190                     | 195                            |
| Same, + $NaNO_3$ ( $NO_3$ 50 p. p. m.).....                                                 | 151                     | 172                            |
| Same, + $Na_2HPO_4$ ( $PO_4$ 50 p. p. m.).....                                              | 219                     | 137                            |
| Same, + $K_2SO_4$ and $NaNO_3$ (K and $NO_3$ each 50 p. p. m.).....                         | 272                     | 193                            |
| Same, + $K_2SO_4$ and $Na_2HPO_4$ (K and $PO_4$ each 50 p. p. m.).....                      | 200                     | 152                            |
| Same, + $NaNO_3$ and $Na_2HPO_4$ ( $NO_3$ and $PO_4$ each 50 p. p. m.).....                 | 254                     | 182                            |
| Same, + $K_2SO_4$ , $NaNO_3$ , and $Na_2HPO_4$ (K, $NO_3$ and $PO_4$ each 50 p. p. m.)..... | 356                     | 149                            |

*Experiment XVIII.*—The effect of pyrogallol upon extract of “exhausted” Miami silt loam is shown by this experiment. The relative results, according to transpiration and green weight, are given in Table XVIII. They show that by the former criterion the pyrogallol appears to be beneficial, while by the latter the growth in

all treatments was practically the same. The general appearance of the plants was in agreement with the transpiration figures. The only general conclusion warranted by such data as these is that the chemical is certainly somewhat beneficial, though to what extent can not be stated.

TABLE XVIII.—*Effect of pyrogallol upon extract of "exhausted" Miami silt loam.*

[P. p. m.=parts per million.]

| Treatment.                                                            | Relative transpiration. | Relative green weight of tops. |
|-----------------------------------------------------------------------|-------------------------|--------------------------------|
| Extract of exhausted Miami silt loam, untreated.....                  | 100                     | 100                            |
| Same, + pyrogallol, 1 p. p. m.....                                    | 110                     | 100                            |
| Same, + pyrogallol, 5 p. p. m.....                                    | 124                     | 94                             |
| Same, + pyrogallol, 10 p. p. m.....                                   | 153                     | 106                            |
| Same, + pyrogallol, 25 p. p. m.....                                   | 193                     | 103                            |
| Same, + pyrogallol, 40 p. p. m., neutralized with trimethylamine..... | 183                     | 106                            |

Of course there are many soils which are unproductive because of the amount and relation of the water and air supply which they afford the plant roots. Thus a sandy soil may be unproductive because it is too well drained and the plants growing therein suffer from lack of water. Also, a heavy clay may be unproductive because it is not adequately drained, the high moisture content so diminishing the supply of air to plant roots that these organs suffer from lack of oxygen and produce a dwarfing effect upon the whole plant. The method of studying soil extracts, rather than the soils themselves avoids these considerations altogether. A soil whose unproductiveness is due to either of the physical conditions just mentioned should give an extract which would produce better plant growth than pure water, for this extract is not influenced by physical properties of the soil from which it was prepared, but is influenced by the chemical properties. It therefore appears that if a soil extract exhibits the unproductiveness of the soil from which it was made the cause of the poor growth obtained lies, to some extent at least, in the chemical properties of the soil solution, rather than in the physical properties of the soil. Whether or not the soils with which this bulletin deals were in good physical condition is not of interest in the present connection. The important facts at the outset are that their aqueous extracts were unproductive and that a comparison of the growth of wheat seedlings in them and in pure water showed that a better growth was always obtained in the latter medium. The last fact proves conclusively that the chemical nature of the extract, and hence the soil solution, was at fault.

There are three possible reasons why a nutrient medium may be unfit for the proper growth of plants: (1) It may be deficient in nutrient salts; (2) it may have too high a concentration of noninju-

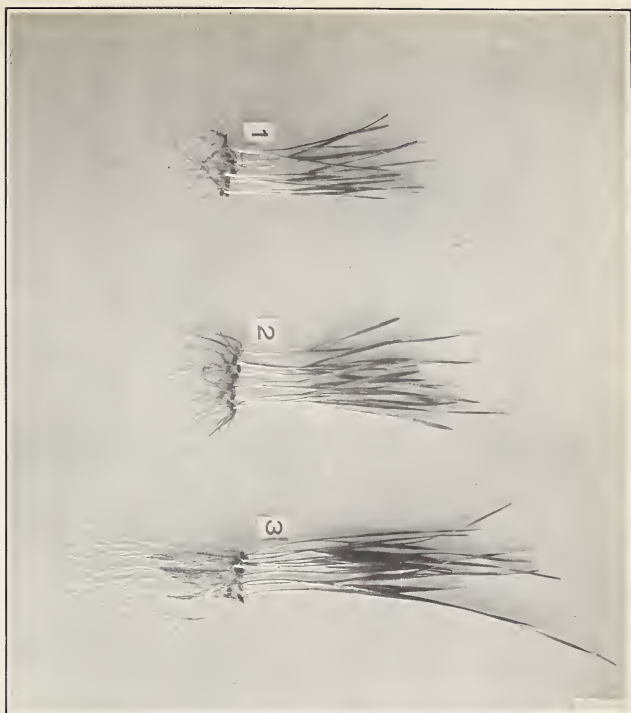


FIG. 1.—WHEAT SEEDLINGS GROWN IN EXTRACT OF DEPARTMENT SOIL WITH STABLE-MANURE TREATMENTS.

[1. Extract of Department soil, untreated. 2. The same plus  $\text{NaNO}_3$  equivalent to nitrogen in manure extract. 3. The same plus manure extract.]

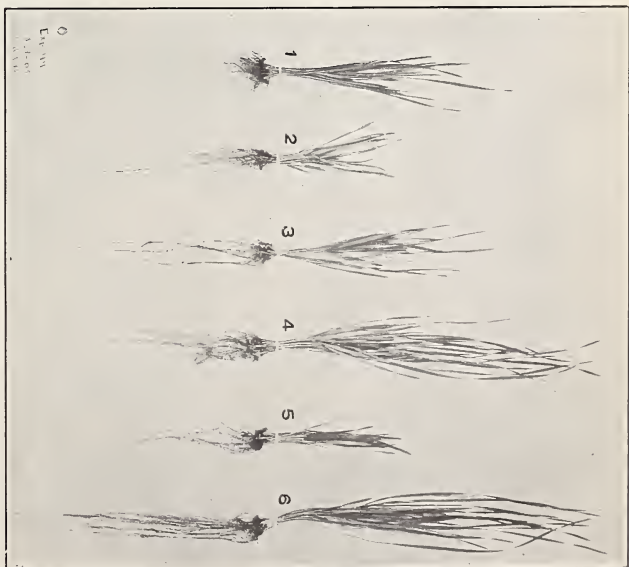


FIG. 2.—WHEAT SEEDLINGS GROWN IN EXTRACT OF DEPARTMENT SOIL WITH DIALYZED STABLE-MANURE EXTRACT.

[1. Extract of Department soil, untreated. 2. The same plus dialyzable portion of manure extract, 1/20. 3. The same plus nondialyzable portion of manure extract, 1/20. 4. The same plus manure extract, original, 1/20. 5. The same plus dialyzed and reuniting manure extract, 1/10. 6. The same plus original manure extract, 1/10.]





FIG. 1.—WHEAT SEEDLINGS GROWN IN NUTRIENT SOLUTIONS WITH DIALYZED STABLE-MANURE EXTRACT.

[1. Knop's solution 48 p. p. m. 2. The same plus dialyzable portion of manure extract, 1/20. 3. The same plus nondialyzable portion of manure extract, 1/20. 4. The same plus manure extract, original 1/20. 5. The same plus dialyzed and renitted manure extract, 1/20.]

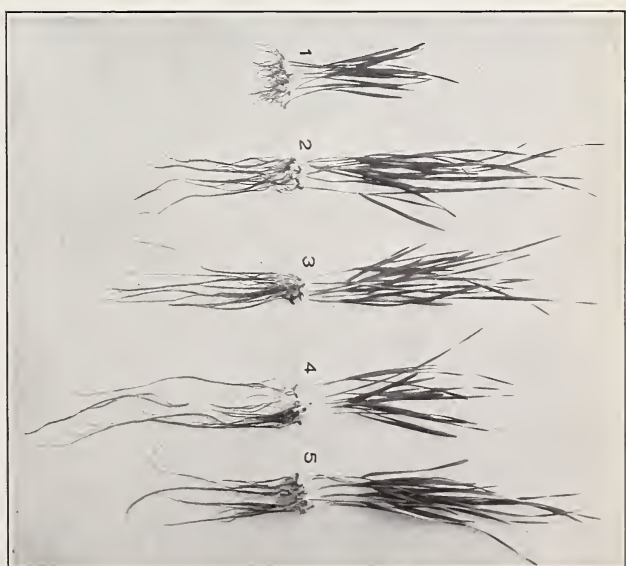


FIG. 2.—EFFECT OF DIALYZABLE AND NONDIALYZABLE PORTIONS OF STABLE-MANURE EXTRACT COMPARED WITH STABLE-MANURE EXTRACT ON DEPARTMENT SOIL EXTRACT. MANURE NATURALLY DIALYZED.

[1. Department soil extract, untreated. 2. Department soil extract plus 1/20 manure extract. 3. Department soil extract plus dialyzable portion of manure extract, 1/20. 4. Department soil extract plus nondialyzable portion of manure extract, 1/20. 5. Department soil extract plus renitted manure extract.]



rious dissolved substances, and (3) it may contain poisonous substances in such concentrations that injury to the plants is actually brought about. That pure water produced a better growth than did the extracts of these soils shows conclusively that the unproductiveness with which we are dealing is caused by either the second or the third of the possible reasons just mentioned. This at once rules out of consideration the explanation of this unproductiveness which should be given according to the prevalent theory of fertilizer practice, namely, that soils which are poor for reasons other than physical ones are lacking in nutrient salts. Furthermore, that the soil extracts here dealt with were markedly improved for the growth of wheat by the addition of nutrient salts shows just as conclusively that they were not unfit for plant growth because of the high concentration. The third possible reason is therefore the only one of the three which is logically possible in the face of the experimental evidence. This conclusion is identical with the one reached from the experiments with absorbing solids.

Additional striking evidence that the beneficial action of nutrient salts on this solution is not primarily due to increase in food materials was obtained when the effects of the nonnutrient salt, sodium chloride, were determined. By addition of this salt<sup>a</sup> an increase in growth of wheat seedlings was obtained in extract of "exhausted" Miami silt loam. Sodium chloride can not be considered as essential for the growth of wheat—indeed the chlorine ion is, if anything, toxic—but in spite of these facts a beneficial effect is brought about. Calcium sulphate was added at the rate of 210 parts per million to this soil extract and it produced a gain in transpiration of 134 per cent and of 39 per cent in green weight of tops. Considering the extremely small amounts of calcium and sulphate ions which are necessary for plant growth it is very improbable that this effect is to be considered as due directly to alteration of the nutritive conditions of the extract. A smaller increase in growth, amounting to 15 per cent by transpiration and 13.5 per cent by green weight, was obtained from the addition of calcium sulphate to extract of Volusia silt loam.<sup>b</sup>

If these extracts contain substances deleterious to plant growth, the question at once arises, How do the various chemicals act in bringing about the marked improvements in growth exhibited in the experiments described above? A priori, there are three ways in which these improvements may be brought about: (1) The salts may cause the plants to be less susceptible to the effects of the injurious materials by increasing the physical concentration of the medium; (2) the

<sup>a</sup>See paper by Wheeler and Breazeale, Eighteenth Ann. Rept. Rhode Island Expt. Sta., 1904-1905, p. 286.

<sup>b</sup>See Bul. 167, Ohio Agr. Expt. Sta., 1905, p. 105.

salts may act chemically as antidotes for the poison, so influencing the metabolism of the plant as to overcome the effects of the injurious substances; and (3) the salts may act in some way upon the toxic bodies themselves, so altering them that they become nontoxic. Whether one or more of these three possibilities actually occur is a difficult question to answer.

Ordinary river water, which often furnishes the supply of cities, is virtually a soil extract, and ordinary distilled water is to be regarded as a distillate from a soil extract. Some very interesting results bearing upon the question just raised were obtained in connection with the work on distilled water presented in the latter part of this bulletin. The salts of Knop's nutrient solution (calcium nitrate 4 parts, potassium nitrate, magnesium sulphate, and dipotassium phosphate, each 1 part) were added to ordinary distilled water in several different concentrations, and then the solution thus prepared was very carefully distilled in glass and this distillate compared with the original water and with the same, plus the salts. Two such experiments may be mentioned.

In the first of these, plants were grown in distilled water, redistilled water, nutrient solution 112 parts per million, and the distillate of the latter; the transpirations of the cultures were, respectively, 100, 243, 723, and 357.

*Experiment XIX.*—This included three different concentrations of the salts. A culture in water redistilled from chromic acid is also included. The relative results are given in Table XIX.

TABLE XIX.—*Effect of salts and of distillation on ordinary distilled water.*

[P. p. m. = parts per million.]

| Treatment.                                                                              | Relative transpiration. |
|-----------------------------------------------------------------------------------------|-------------------------|
| Ordinary distilled water.....                                                           | 100                     |
| Same, redistilled.....                                                                  | 169                     |
| Same, with addition of potassium dichromate and sulphuric acid and then redistilled ... | 227                     |
| Same, with addition of salts, 112 p. p. m., and redistilled.....                        | 254                     |
| Same, with addition of salts, 445 p. p. m., and redistilled.....                        | 227                     |
| Same, with addition of salts, 890 p. p. m., and redistilled.....                        | 323                     |

From both of these tests it appears that the injurious materials of the ordinary water are less volatile with steam in the presence of the salts than in their absence. The effect produced by distilling from this salt solution is very similar to that produced by distilling from potassium dichromate and sulphuric acid or from either of these chemicals alone. Just what this action may be it is impossible to determine at present.

The effect of pyrogallol and hydroquinone upon these soil extracts can be explained on the ground that they react chemically with the toxic substances, or that they so affect the plant as to make it less

susceptible to the action of these bodies. At present we are unable to go further in this regard. The effects of several other chemicals will be discussed in the following chapter.

#### SOME PROPERTIES OF THE TOXIC SUBSTANCES OF UNPRODUCTIVE SOILS.

Since the toxic bodies here dealt with exist in the soil extract in presumably small quantities, it has been thus far impossible to separate them and study them chemically, though it is to be hoped that this will eventually be accomplished. The considerations to be given in this chapter are, therefore, not of the nature of direct chemical evidence, but are inferences drawn from the behavior of the toxic bodies as they effect wheat seedlings grown in the soil extracts. The following questions regarding the nature of the toxic bodies will be discussed: (a) Solubility, (b) volatility, (c) acidity, (d) whether they are organic, and (e) their power of being absorbed.

*Solubility.*—That the deleterious bodies present in the soils here studied are either very slightly soluble or are present in the soil in exceedingly minute quantities is made apparent by the small amounts which are to be found in the aqueous extracts. Evidence of this was obtained by adding soil to Knop's nutrient solution (the form described in Bulletin 28), in the same way as ferric hydrate and carbon black were added to the soil extract, and then growing plants in these solutions, comparison being made with the untreated nutrient medium. As has been shown in Bulletin 28 and by Breazeale, the addition of absorbing solids to a nutrient solution has a very marked accelerating influence on wheat seedlings grown therein, and it was found in the test just mentioned that the various soils have a similar effect. An experiment in this regard will be presented here.

*Experiment XX.*—In this experiment were used Cecil clay, "poor," Leonardtown loam, "good" and "poor,"<sup>a</sup> Hagerstown clay, Takoma lawn soil, and Department soil, a small quantity of each soil being added to a nutrient solution containing 112 parts per million of total salts. The relative growths, by transpiration and green weight, are given in Table XX.

TABLE XX.—*Effect of adding soils to nutrient solution.*

| Treatment.                                   | Relative transpiration. | Relative green weight of tops. |
|----------------------------------------------|-------------------------|--------------------------------|
| Nutrient solution.....                       | 100                     | 100                            |
| Same, + Cecil clay, poor.....                | 126                     | 108                            |
| Same, + Cecil clay, poor, subsoil.....       | 116                     | 95                             |
| Same, + Leonardtown loam, poor, subsoil..... | 107                     | 100                            |
| Same, + Leonardtown loam, good.....          | 116                     | 103                            |
| Same, + Leonardtown loam, good, subsoil..... | 133                     | 112                            |
| Same, + Hagerstown clay.....                 | 119                     | 107                            |
| Same, + Hagerstown clay, subsoil.....        | 147                     | 129                            |
| Same, + Takoma lawn soil.....                | 111                     | 108                            |
| Same, + Department soil.....                 | 137                     | 121                            |

<sup>a</sup> On these soils see Bul. 23, and Livingston, B. E., Relation of Transpiration to Growth in Wheat, Bot. Gaz., 40, 178-195 (1905).

From the above table it is clear that all of the soils have produced a beneficial effect, apparently regardless of the agricultural productiveness of the soils used. The explanation of this fact is probably that offered in Bulletin 28 and by Breazeale for the effect of adding absorbing solids to a nutrient solution, namely, that the wheat seedlings produce in the medium substances toxic to themselves, and that these are removed from solution by the absorbents—in this case the added soils—thus diminishing or preventing their retarding action. The property of absorbing substances from solution is one possessed to a greater or less extent by all finely divided solids, and, as has been pointed out (see Bulletin 30), the soil is usually a very good absorber. It appears probable that in the experiment just described the addition of soil to the nutrient solution tended toward two quite opposite effects. First, it acted as an absorbent, as does carbon black, and it tended to accelerate growth by removing toxic bodies arising from the growth of the seedlings used in the cultures. Second, in so far as the soil itself contained soluble toxic bodies these tended to retard growth. The results of two such tendencies should, of course, be their algebraic sum, and if, as appears to have been the case, the latter tendency were of low while the former was of high value, the observed effect upon the plants should be one of accelerated growth. This is unquestionably the case in this experiment, which seems to indicate quite clearly that the retarding action due to the presence of water-soluble toxic bodies in the soil was relatively small and was more than counterbalanced by the absorbing action. It logically follows that this low value of the retarding influence is to be considered as due to the fact that the small amounts of soil used either contained only traces of deleterious matter or that the latter was soluble to only a slight degree.

*Volatility.*—The question of the volatility of the toxic substances here considered was attacked by boiling and distilling the soil extract and also by concentrating it on the water bath. If the toxic bodies were volatile with steam, boiling the extract should improve it, for these bodies should be, in part, at least, removed by the boiling. If they were volatile and not destroyed at the temperature of boiling water, distilling the extract should make the distillate less fitted for plant growth, for the toxic bodies should then be removed from the retort and condensed in the receiver. If the toxic bodies were not volatile and not destroyed by boiling they should be concentrated in the retort, so that the residue therefrom should be more injurious to the seedlings than the original extract, unless, indeed, the increased concentration in salts brought about by this process should counteract the effect of the greater concentration in the toxic bodies.

A comparison was made between the growths obtained in extract of Takoma lawn soil, untreated, and in the same extract concentrated,

by boiling, to one-tenth of its original volume. The plants grown in the concentrated extract gave a transpiration of 83, as compared with 100 for the untreated extract.

The effect of boiling the extract of Takoma lawn soil was compared with that of distilling the same. Plants grown in the extract boiled five minutes gave a transpiration of 151, those in the distillate of the extract 257, as against 100 for the untreated extract. These figures point toward the conclusion that boiling the extract decreases its toxic action. This may mean that the injurious bodies are altered by boiling so as to become less toxic but not nontoxic, and this may explain why a greater increase in toxicity was not observed in the case of the concentrated extract used above. Of course it must be remembered in this connection that it is highly probable that we are dealing with several deleterious substances and that any single treatment of the soil extract may correct one or more of these.

The question relating to the volatility of the injurious bodies in the other soils has not been so thoroughly studied. Experiments to test the effect of boiling and distilling extract of Department soil brought out the fact that boiling seems to be more beneficial than distilling, though the latter produced a marked effect in growth, as has been already shown.

The following test brings out the point just mentioned in regard to Department soil: One portion of the extract was boiled for five minutes and another portion distilled in glass. The plants in the former cultures transpired 316, in the latter 280, as against 100 in the untreated extract.

The condition of affairs indicated by the above figures may be explained by supposing the process of boiling to remove or change the toxic action of this extract, the toxic materials being perhaps partly volatile with steam.

*Experiment XXI.*—With the same soil extract tests were made to determine whether or not the action of sodium nitrate, already described, could be regarded as in any way replaceable by that of boiling. The salt was added to the extract and a portion of the resulting solution was then boiled five minutes, after which wheat seedlings were grown in the usual way in the untreated and in the boiled and unboiled portions of the treated extract. The relative growths, by transpiration, green weight, and dry weight, are given in Table XXI. The sodium nitrate was added in several concentrations.

TABLE XXI.—*Effect of boiling upon extract of Department soil plus sodium nitrate.*

[P. p. m.=parts per million.]

| Treatment.                                                   | Relative transpiration. | Relative green weight. | Relative dry weight. |
|--------------------------------------------------------------|-------------------------|------------------------|----------------------|
| Extract of Department soil, untreated.....                   | 100                     | 100                    | 100                  |
| Same, boiled 5 minutes.....                                  | 165                     | 137                    | 111                  |
| Same, + 50 p. p. m. $\text{NaNO}_3$ .....                    | 142                     | 128                    | 106                  |
| Same, + 50 p. p. m. $\text{NaNO}_3$ , boiled 5 minutes.....  | 256                     | 171                    | 136                  |
| Same, + 100 p. p. m. $\text{NaNO}_3$ .....                   | 138                     | 147                    | 125                  |
| Same, + 100 p. p. m. $\text{NaNO}_3$ , boiled 5 minutes..... | 229                     | 151                    | 114                  |
| Same, + 200 p. p. m. $\text{NaNO}_3$ .....                   | 138                     | 153                    | 119                  |
| Same, + 200 p. p. m. $\text{NaNO}_3$ , boiled 5 minutes..... | 246                     | 179                    | 135                  |
| Same, + 400 p. p. m. $\text{NaNO}_3$ .....                   | 155                     | 151                    | 113                  |
| Same, + 400 p. p. m. $\text{NaNO}_3$ , boiled 5 minutes..... | 191                     | 165                    | 119                  |

With one exception the data of the above table all agree in showing that the accelerating influence of sodium nitrate is increased when the treated extract is boiled. This seems to indicate that the effect of the salt is not brought about through the same channels as that of the boiling process. Perhaps the retarding influence of a portion of the toxic bodies may be counteracted by the salt and that of another portion by boiling.

In the case of the Volusia silt loam no marked effect in either direction was observed when the extract was concentrated to one-tenth of its original volume. This may mean that the toxic materials there present are partly volatile and were not concentrated with the extract. It is also possible, though hardly probable, that the increased concentration of the contained salts which accompanies the increased concentration of the toxic bodies offsets the retarding effect of the latter.

In some investigations carried on by this Bureau in cooperation with the Cornell Experiment Station, at Ithaca, N. Y., it was found that the extract of Dunkirk clay loam contains toxic bodies which seem to be almost or quite completely volatile with steam, so that boiling the extract results in a great increase in growth, while the distillate retains the toxic properties. The residue from distillation in glass is always much better for the growth of seedlings than is the distillate.

*Acidity.*—It has long been supposed by workers in agriculture that the unproductiveness of many poor soils is due to acidity, such soils being spoken of as “acid” or “sour.” It might be expected, therefore, that the toxic substances here considered are acid in their nature, and that the toxic properties are due to the presence of free acids. It is to be remembered, however, that the toxic bodies may be acid in their reaction and yet this acidity may not be the cause of their deleterious effects upon the plants. Such instances are common in the literature of chemical stimulation. An example may be taken from the case of hydrocyanic acid, a very powerful poison. If the toxicity of this chemical to plants and animals were due to the hydro-

gen ion—i. e., to its acidity—this substance should be no more toxic than nitric or hydrochloric acid. Since it is vastly more poisonous, it follows that the acidity of the poison is not the main factor in its retarding action, but that the cyanogen is the active agent. This conclusion is supported by the fact that potassium or sodium cyanide possesses about the same toxic properties as the acid and yet has no free hydrogen ions. It thus appears possible that the toxic bodies of these poor soils may be acid in their reaction and yet this acidity may have practically nothing to do with their toxic action. This question has been studied experimentally, and the available evidence will be given here.

Concerning Takoma lawn soil, it is shown in Bulletin 28 that the extract is acid in its reaction to litmus and phenolphthalein, but neutral to methyl orange. Its acidity amounts to from two sixteen-thousandths to four sixteen-thousandths normal with phenolphthalein as indicator and N/100 potassium hydrate as standard alkali. It was shown (Bul. 28, p. 32) in experiments upon the soil itself that the order of the beneficial effects derived from various treatments did not follow at all the order of the effect of those treatments upon the acidity of the soil extract. This is conclusive proof that in the case of this soil the main factor which tends to retard plant growth is not the hydrogen ion.

The same question was attacked by adding acids to the extract of Takoma lawn soil. If acidity were the cause of the poor growth obtained in this soil, an increase in acidity should markedly decrease the growth; but if other factors were active in causing the poor growth, such an increase in acidity might either increase or decrease the growth or fail to affect it at all.

*Experiment XXII.*—In this experiment sulphuric and hydrochloric acids were added to the soil extract. The concentrations used are stated in terms of normal acid. The relative results, according to transpiration, are given in Table XXII.

TABLE XXII.—*Effect of sulphuric acid and hydrochloric acid upon extract of Takoma lawn soil.*

| Treatment.                                       | Relative transpiration. |
|--------------------------------------------------|-------------------------|
| Soil extract, untreated.....                     | 100                     |
| Same, + $\text{H}_2\text{SO}_4$ , N/70,000.....  | 88                      |
| Same, + $\text{H}_2\text{SO}_4$ , N/100,000..... | 117                     |
| Same, + $\text{HCl}$ , N/20,000.....             | 104                     |
| Same, + $\text{HCl}$ , N/30,000.....             | 141                     |
| Same, + $\text{HCl}$ , N/40,000.....             | 110                     |
| Same, + $\text{HCl}$ , N/50,000.....             | 124                     |

From Table XXII it appears that a slight increase in the acidity of the soil extract does not decrease growth but increases it slightly.

*Experiment XXIII.*—In this experiment the effect of acetic acid in the same extract was tried, with results similar to the last. The relative transpiration figures are given in Table XXIII.

TABLE XXIII.—*Effect of acetic acid on extract of Takoma lawn soil.*

| Treatment.                         | Transpiration. |
|------------------------------------|----------------|
| Soil extract, untreated.....       | 100            |
| Same, + acetic acid, N/1,000.....  | 92             |
| Same, + acetic acid, N/5,000.....  | 101            |
| Same, + acetic acid, N/10,000..... | 85             |
| Same, + acetic acid, N/15,000..... | 110            |
| Same, + acetic acid, N/20,000..... | 103            |

Still another way of attacking this problem is to add the salts of a nutrient solution to the soil extract and to compare the growth therein with that in a similar nutrient solution prepared with pure water, the latter with and without addition of acid to make its acidity equal to that of the soil extract plus the salts.

*Experiment XXIV.*—Hydrochloric, nitric, sulphuric, phosphoric, acetic, and oxalic acids were used. The salts used were in amount sufficient to make a solution of 75 parts per million. The relative transpiration figures are given in Table XXIV.

TABLE XXIV.—*Comparison of soil extract to which has been added the nutrient salts with similar nutrient solution with and without the addition of acids.*

[P. p. m.=parts per million.]

| No. | Treatment.                                                                  | Relative transpiration. |
|-----|-----------------------------------------------------------------------------|-------------------------|
| 1   | Extract of Takoma lawn soil + nutrient salts, 75 p.p.m.....                 | 100                     |
| 2   | Nutrient solution, 75 p.p.m.....                                            | 224                     |
| 3   | Same, + HCl, acidity equal to that of (1) .....                             | 173                     |
| 4   | Same, + HNO <sub>3</sub> , acidity equal to that of (1) .....               | 109                     |
| 5   | Same, + H <sub>2</sub> SO <sub>4</sub> , acidity equal to that of (1) ..... | 203                     |
| 6   | Same, + H <sub>3</sub> PO <sub>4</sub> , acidity equal to that of (1) ..... | 191                     |
| 7   | Same, + acetic acid, acidity equal to that of (1) .....                     | 184                     |
| 8   | Same, + oxalic acid, acidity equal to that of (1) .....                     | 216                     |

It is evident that none of the acids used had as marked depressing effect upon growth as the toxic bodies of the soil extract. None of them, in this concentration, produced any increase in growth over the nutrient solution. Some retardation is apparent in the case of all the acids used, nitric showing the greatest effect and oxalic the least, but none of these exhibits as great a retarding effect as do the toxic bodies derived from the soil. It is pertinent to note here that the acids of the soil are more apt to be of an organic than of an inorganic nature, so that these results must be considered as strong additional evidence that the toxic bodies of Takoma lawn soil are active through some other property than their acidity.

The extract of Department soil is even less acid than that of Takoma lawn soil. No experiments relating to this acidity have thus far been performed.

From Bulletin 167 of the Ohio Agricultural Experiment Station we derive the following facts regarding the acidity of Volusia silt loam from Wooster. The soil extract is slightly acid to phenolphthalein and to litmus. Sulphuric acid was added to this soil in the proportion of 250 parts per million and produced a beneficial effect of 4.9 per cent by transpiration and 12.5 per cent by green weight. It seems evident, therefore, that the deleterious substances in this soil can not be considered as primarily active through the hydrogen ion, the same conclusion as was reached in the case of Takoma soil.

As has been shown on preceding pages, all of the soil extracts studied have been found to be greatly benefited by the treatment with carbon black. A comparison of the acidity of the treated and untreated soil extract in these cases showed no decrease in this property after the treatment. This is general evidence that the toxicity, which is removed by the absorbing solids, is not always due to the presence of free hydrogen ions.

Furthermore, all of the extracts studied, as well as the soils themselves, have been markedly improved by treatment with calcium carbonate. This salt, being only slightly soluble, may be beneficial, either through its absorbing action or through the dissolved portion which is added to the extract. The dissolved portion may have an accelerating action (1) through the nutrient value of calcium (which is improbable on account of the small amounts of this element necessary for the development of most plant species); (2) through some physiological property of this salt by which it counteracts the effect of the poisons, and (3) through some effect of calcium carbonate upon the toxic materials themselves. Evidence is presented in Bulletin 28 that, for Takoma lawn soil, either the second or third supposition must be regarded as the true one. If it be the third, it might be supposed, as has commonly been done by agriculturists working with lime, that it owes its effect mainly to its alkalinity, which enables it to neutralize the acidity of the soil extract. But it has been shown also in Bulletin 28 that other alkalies, such as sodium carbonate and sodium hydrate, do not have nearly so great an effect upon extract of Takoma lawn soil as does calcium carbonate. This fact makes it appear that the action of this salt takes place through some other channel, and also furnishes evidence that the toxic bodies are not mainly active through their acidity. Considerable further study of the effect of calcium carbonate will be necessary before any satisfactory conclusions as to its mode of action can be reached.

*Organic or inorganic nature of these toxic substances.*—The question as to whether the toxic bodies of these soils are organic or inorganic

has been studied only for Takoma lawn soil. The evidence is given in Bulletin 28, page 29. It is there shown that if an aqueous extract of this soil is evaporated to dryness, the latter charred to decompose organic matter, and then taken up again in pure water, an increase in transpiration of 25 per cent is obtained, due to the evaporating and charring. Since the inorganic salts, aside from the nitrates, are not changed by this treatment, the conclusion is indicated that the deleterious properties of this extract are organic in their nature.

*Power of being absorbed.*—From the beneficial effects produced by means of treatment with absorbing solids upon soil extracts here studied, it is clear that, whatever may be the nature of the toxic bodies, they are capable of being absorbed by these solids, a property, shared by many organic and some inorganic compounds.

Summing up the various fragmentary lines of evidence on the nature of the toxic substances so far studied, we may say that they appear to be slightly soluble in water; that they are volatile with steam in the case of some soils and nonvolatile in others; that they are usually accompanied by an acid reaction of the extract, but it is quite certain that, in the cases studied, the hydrogen ion is not the direct cause of their toxicity; that they are probably organic in their nature; and that they are absorbed by finely divided solids in much the same way as organic dyes, etc.

#### ORIGIN OF THE TOXIC SUBSTANCES.

On the origin of the deleterious bodies of poor soils the evidence is as incomplete as that on their nature, as would be expected from the newness of this whole line of inquiry. The work of the Duke of Bedford and Pickering, reviewed above under the heading Literature, makes it clear that, in some cases at least, the growth of grasses causes the appearance in the soil of substances injurious to fruit trees. The paper of Woods on mosaic disease of tobacco already cited, points out that the oxydase which seems to favor the disease probably originates from tobacco plants. The experiments of Livingston with bog water, also mentioned in the consideration of the literature, seems to indicate that the anaerobic fermentation of undrained swamps develops substances which have a retarding effect on plant growth. The experiments of Macaire and the other early writers are to be considered as inconclusive. The work of the present authors in respect to the origin of bodies toxic to plants have been directed mainly along two lines, first to determine whether or not wheat plants give rise to materials toxic to themselves, and, second, to find whether the exudations from germinating wheat seeds are deleterious to the growth of the seedlings. Some attention has been given to the question of the possible origin of some of these injurious bodies from trees. The evidence so far obtained will be given here.

In Bulletin 28 (p. 34) an experiment is described in which the effect of adding ferric hydrate to clean glass sand was shown to be an increase in transpiration of the plants amounting to 31 per cent. It was also shown that in this experiment there was no increase in water soluble salts in the sand, owing to the presence of the ferric hydrate. The explanation offered for these results is that the roots of the seedlings produce material injurious to themselves and that in the presence of the absorbing solid this material is removed from solution as fast as formed and hence fails to be injurious.

It was also shown in the same bulletin (p. 35) that a used nutrient solution which has reached the point of "exhaustion," where the growth of seedlings in it is exceedingly poor, is greatly improved (53 and 63 per cent by transpiration) by the addition of ferric hydrate or carbon black. Since the original nutrient solution was prepared from pure water and from chemically pure salts it appears that the only available explanation of this fact is by assuming injurious bodies to arise in it during the growth of the first set of plants. By removing these bodies by means of the absorbing solids a better growth should be obtained, as is actually the case.

In the same bulletin it is further brought out that ferric hydrate and carbon black, when added to the nutrient solution at the first planting, give a much better growth (33 and 27 per cent gain in transpiration) than is exhibited by cultures in the same solution without the solids.

A number of other experiments on nutrient solution have been performed and will now be presented.

*Experiment XXV.*—This experiment was carried out to determine the relative effects of the addition of ferric hydrate to nutrient solutions of two different salt contents, 4 and 20 parts per million of total salts. The relative transpiration figures are given in Table XXV. The growth in each untreated nutrient solution is considered as 100.

TABLE XXV.—*Effect of ferric hydrate added to nutrient solution.*

| Treatment.                          | Relative transpiration. |
|-------------------------------------|-------------------------|
| Nutrient solution, 4 p. p. m. ....  | 100                     |
| Same, + ferric hydrate .....        | 165                     |
| Nutrient solution, 20 p. p. m. .... | 100                     |
| Same, + ferric hydrate .....        | 133                     |

It is seen from these figures that the absorbing solid has been markedly beneficial on both solutions, but that its effect was greater on the weaker one. This suggests that injurious materials are produced by the growth of the plants and that the effect of these is decreased, but not entirely overcome, in the presence of the larger amount of salts. If the salts correct a portion, but not all, of the injurious property, then the addition of ferric hydrate to the stronger

solution should not produce such a marked effect as in the case of the weaker one. This is exactly what occurred.

*Experiment XXVI.*—In this test a nutrient solution containing 70 parts per million was prepared, cultures were set up in three portions of it, and were treated in three different ways. In the first case nutrient salts to the amount of 10 parts per million were added every four days, in the second the entire solution was replaced by a fresh one of the same kind every four days, and in the third ferric hydrate was added to the solution at the start and nothing further was done, excepting to add pure water as it was lost by transpiration. The relative transpiration figures are given in Table XXVI.

TABLE XXVI.—*Effect of adding salts, changing solution, and presence of ferric hydrate.*

| Treatment.                                          | Relative transpiration. |
|-----------------------------------------------------|-------------------------|
| Nutrient solution, salts added every four days..... | 100                     |
| Same, changed every four days.....                  | 109                     |
| Same, + ferric hydrate, not changed.....            | 129                     |

From the fact that changing the solution was more beneficial than adding the salts it is to be concluded either that injurious substances were forming in the solution during the growth of the plants or that the salts added were not in a large enough amount to replace what had been absorbed. The latter supposition is improbable from our general experience with plant growth, and the former is strengthened very much by the fact that without change of solution the presence of ferric hydrate had the most marked effect of all three treatments. It appears, then, that the effect of the injurious bodies assumed to be produced in the solution during the growth of the cultures was partially overcome by changing the solution, but that the changes were not made often enough to remove that effect altogether, while the presence of ferric hydrate, which should absorb the injurious materials as fast as produced, overcame practically all of their effect.

From all of the experimental data given it seems almost certain that injurious bodies do appear in the solution and in sand during the growth of wheat seedlings. Whether or not these arise directly from the roots of the seedlings or are indirectly produced from the interaction of the roots with bacteria is a question which has not yet been attacked.

When seeds of wheat are soaked preparatory to germination it is observed that the water in which they lie is unsuited to the growth either of the same or of other seedlings. This has led to some preliminary tests in which it was brought out that not only organic matter but also nutrient salts escape from the imbibing seed. It appears probable that some of the organic matter is injurious. This is a field which deserves further investigation.

In studying the Takoma lawn soil it was suggested that the trees growing in the lawn at Takoma Park, Md., from which this soil was obtained, might have given rise to some of the injurious materials present in the soil. Experiment XXVII was carried out to test this question.

*Experiment XXVII.*—Water was thrown as a spray upon the bark of oak, chestnut, and pine trees and the run-off was carefully collected and added to extract of Takoma lawn soil to determine whether or not it might contain injurious bodies. At the same time leaves from these trees were washed in water and this water used in the same way. The relative transpiration figures are given in Table XXVII.

TABLE XXVII.—*Effect of bark and leaf washings upon extract of Takoma lawn soil.*

| Treatment.                                | Relative transpiration. |
|-------------------------------------------|-------------------------|
| Extract of Takoma lawn soil.....          | 100                     |
| Same, + washing from oak bark.....        | 59                      |
| Same, + washing from chestnut bark.....   | 79                      |
| Same, + washing from pine bark.....       | 76                      |
| Same, + washing from oak leaves.....      | 75                      |
| Same, + washing from chestnut leaves..... | 71                      |
| Same, + washing from pine leaves.....     | 36                      |

From these data it appears that these different washings had a considerable effect upon the plants, and it seems reasonable to suppose that a portion at least of the injurious material of this soil may have had its origin in some such manner as this—from the trees which occupy the ground. No further work has so far been done upon this question.

Briefly summarizing the evidence presented on the origin of the toxic bodies: Such bodies are apparently produced in soil and extract from the growth of wheat seedlings. Similar bodies appear to diffuse out of soaking wheat seeds, and substances of similar physiological action appear to be washed from the leaves and trunks of trees. They may be, and probably are in many cases, the products of the growth of bacteria and fungi.

If substances injurious to growth are excreted from the roots of growing plants the suggestion at once arises that a great many plant responses to chemicals hitherto considered as stimulation phenomena may be due either to the correction by the chemical of injurious bodies in the medium, or to an effect upon the roots which results in a decreased amount of toxic bodies excreted. A discussion of this interesting suggestion would lead us into the province of chemical stimulation, and thus far afield from the purposes of the present publication.

## SUMMARY.

The main conclusions indicated by the experiments described above are as follows: That toxic substances exist in these soils is apparent from several different lines of evidence. (1) Pure water is uniformly better suited to growing wheat seedlings for short periods than is the soil extract from the poor soils. It is also better suited to the preparation of a nutrient solution. (2) Finely divided, insoluble solids, when shaken with these extracts and then removed by filtering, generally produce a great improvement. Since the only conceivable way in which these can act is through their absorbing action, it is to be supposed that they owe their effect to the fact that they absorb, and thus remove from solution, the toxic bodies existing in the extract. (3) The extracts of these soils are benefited by nutrient salts, especially sodium nitrate, but it is pointed out that these may be effective through some other means than the phenomena of nutrition. Certain nonnutrients, such as sodium chloride, pyrogallol, and tannic acid, produce marked beneficial results. Since these can not act directly through nutrition, they must have some action either upon the toxic bodies, making them nontoxic, or upon the plant, causing it to become more or less immune to the toxic bodies. (4) The same chemicals have similar effects when added to the soil itself. Here the action is more difficult of analysis, but it seems probable that the chemicals act in the same general way as in the aqueous extract.

The toxic substances here considered appear to be but slightly soluble in water; they are sometimes volatile with steam and sometimes not; the soil extracts containing them are usually more or less acid in reaction, but the acidity is not in itself the cause of their deleterious action; they are probably organic, and are absorbed by finely divided solids.

Evidence is presented to the effect that injurious materials similar to the ones existing in the soils are produced by the growth of wheat in water or sand cultures. Bodies of similar toxic action are also exuded from soaking wheat seeds, and, in some cases at least, may be washed from the bark and leaves of trees.

**EFFECT OF ORGANIC SUBSTANCES AS FERTILIZERS.**

## INTRODUCTION.

Owing to the exceedingly great complexity of such substances as stable manure and green manure, it has been so far practically impossible to determine exactly the manner in which these act upon the soil and upon the plant to produce the improvement in the growth

of the latter which commonly follows their use. A step toward the solution of this problem has apparently been made by means of the experiments here described. These experiments were mainly performed upon Takoma lawn soil. Several tests were made with the subsoil from the grounds of the Department of Agriculture at Washington, which has already been described. The methods were those used in paraffined wire pot or bottle cultures. "Chul" wheat was grown in the cultures, thirty plants comprising each cultural unit, and the experiments were run usually twelve to sixteen days. The materials studied were the two commonly used organic fertilizers—fermented stable manure and green manure—the latter in the form of the leaves of clover plants. Since the addition to the soil of either stable manure or green manure alters to a large extent its physical condition, most of these tests were made with aqueous solutions of the fertilizing materials rather than with the substances themselves, thus largely avoiding the consideration of their physical effects upon the soil.

An aqueous extract of stable manure was prepared, filtered, and allowed to stand in two loosely stoppered glass containers until fermentation had ceased. The resulting solution in one of the vessels contained no nitrates, while that in the other contained nitrates corresponding to about 300 parts per million of  $\text{NO}_3$ . The reason why nitrates should have been present in one lot of material and not in the other was not determined. The final difference between these two separate fermentations of the extract is interesting in illustrating how complex is the problem involved in the ripening or fermenting of stable manure. The extract containing no nitrates will be designated as No. 1, the other as No. 2. Extract No. 3, which was used in bottle cultures, was made at another time, and was found to contain, after fermentation had ceased, about 200 parts per million of nitrates ( $\text{NO}_3$ ) and 625 parts per million of nitrites ( $\text{NO}_2$ ).

The green leaves of clover were finely ground and the juice expressed, the residue being washed several times and again pressed, so as to obtain in the solution the maximum amount of the soluble materials. In one series of experiments this was done immediately before the juice was to be used, so as to avoid fermentation as far as possible. The material remaining after expressing the juice was in some cases used in comparison cultures. Another series of tests was carried out, using dried clover juice. This was prepared by exposing the expressed juice in shallow porcelain plates to the action of the wind and sunshine until it had evaporated to dryness. No fermentation was observed during this process.

## EXPERIMENTS WITH MANURE EXTRACT.

Comparison, by means of the method of paraffined wire pots, of the effects produced upon Takoma soil by the addition of the two different manure extracts in the proportion of 75 c. c. to 2,000 grams of soil showed that extract No. 1 produced an increase in growth of 26 per cent by transpiration and 32 per cent by green weight, while extract No. 2 gave an increase of 54 per cent by the former criterion and 49 per cent by the latter.<sup>a</sup> It thus appears that while extract No. 1 produced a marked increase, the other extract is nearly twice as effective on this soil. This would seem to indicate that the presence of nitrates in extract No. 2 probably explains its greater effectiveness, but it is evident that nitrates alone can not explain the entire action, since extract No. 1, containing no nitrates, has a marked beneficial effect.

To gain evidence regarding the relative effectiveness upon this soil of the inorganic salts and of the organic matter in extract No. 2, three samples of 75 c. c. each were evaporated to dryness in porcelain dishes on the water bath and the residue burned. The alkaline ash was neutralized with hydrochloric, sulphuric, and nitric acids, respectively. The ash was then taken up in distilled water and used upon 2,000 grams of soil. The solution put into the soil was strictly neutral in its reaction. Wheat cultures were then made in the soils so treated and compared with an untreated soil, together with the soil treated with 75 c. c. of the original manure extract.

The results of this experiment, repeated several times, showed that the ash as chlorides caused a slight depression in transpiration and the ash as sulphates a very slight increase. The ash as nitrates had a beneficial effect about equal to that obtained by the use of the original extract, which, of course, contained less nitrates than when all of the ash is in the form of nitrates, the increase being about 50 per cent in transpiration and about 75 per cent in green weight. It seems from these results that the organic matter of the manure extract, possibly together with ammonium salts which were lost by incineration, probably plays an important rôle in the beneficial action of the manure extract. A discussion of the relative values of the ammonium salts and the organic matter will be presented later.

The effect of manure extract was also studied by means of bottle cultures. It has been shown<sup>b</sup> that the good and poor qualities of soils are usually transmitted to their aqueous extracts, and that treatments which are found to be beneficial upon the soils are also beneficial in the

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<sup>a</sup> On the use of transpiration as a criterion for judging the relative growth of wheat in such cultures, see Livingston, B. E., *Relation of Transpiration to Growth in Wheat*, Bot. Gaz. **40**, 178-195 (1905).

<sup>b</sup> Buls. 23 and 28, Bureau of Soils, U. S. Dept. of Agr.

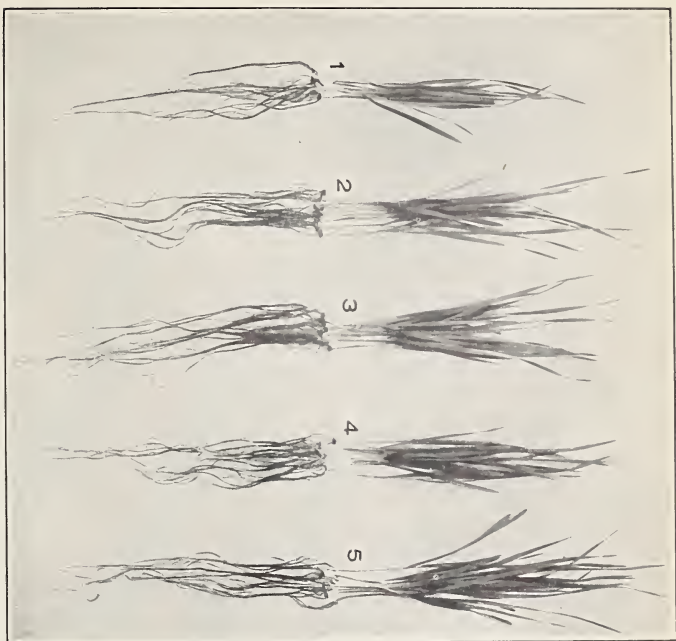


FIG. 1.—EFFECT OF DIALYZABLE AND NONDIALYZABLE PORTIONS OF STABLE-MANURE EXTRACT COMPARED WITH STABLE-MANURE EXTRACT ON KNOP'S SOLUTION. MANURE NATURALLY DIALYZED.

[1. Knop's solution, untreated. 2. Knop's solution plus 1/20 manure extract. 3. Knop's solution plus dialyzable portion of manure extract, 1/20. 4. Knop's solution plus nondialyzable portion of manure extract, 1/20. 5. Knop's solution plus re-untreated manure extract.]

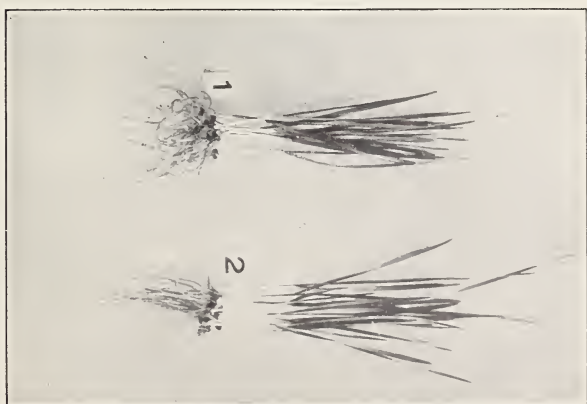
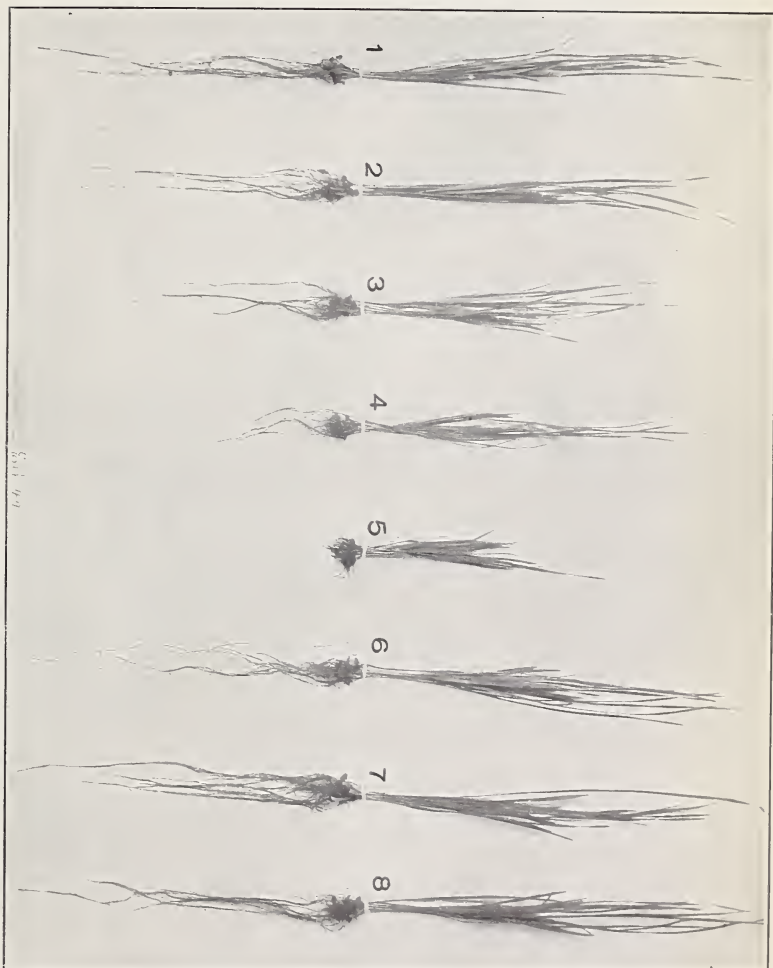


FIG. 2.—WHEAT SEEDLINGS GROWN IN DISTILLED WATER.

[1. Water simply redistilled in glass. 2. The same water boiled five minutes.]





WHEAT SEEDLINGS GROWN IN DISTILLED WATER TREATED WITH QUARTZ FLOUR, FERRIC HYDRATE, AND CARBON BLACK.

[1. Redistilled water treated with quartz flour. 2. Redistilled water treated with ferric hydrate. 3. Redistilled water treated with carbon black. 4. Redistilled water untreated. 5. Ordinary distilled water untreated. 6. Ordinary distilled water treated with carbon black. 7. Ordinary distilled water treated with ferric hydrate. 8. Ordinary distilled water treated with quartz flour.]



extracts. The soil extract used was made by the method described in Bulletins 23 and 28 of this Bureau. In these experiments the subsoil from the grounds of the Department of Agriculture at Washington was used with manure extract designated above as No. 3. It was found at the start that the manure extract was most beneficial upon this soil extract when used in the proportion of 1 part by volume to 19 parts of the soil solution. Therefore this concentration was chosen for the work.

The first experiment consisted in a comparison of the effects of the original manure extract with those of its ash, the alkalinity of the latter being corrected as above by means of sulphuric or nitric acid. The criteria for judging of the relative growth of the plants were total transpiration, green weight, and dry weight of the tops. The plants were grown in two darkened bottles, each having a capacity of 240 c. c. The results are given in Table XXVIII. A photograph of the plants at the end of the experiment is shown in Plate I.

TABLE XXVIII.—*Comparison of the effect of manure extract and ash from the same on extract of Department soil.*

[Plate I.]

| No. | Treatment.                                             | Relative transpiration. | Relative green weight. | Relative dry weight. |
|-----|--------------------------------------------------------|-------------------------|------------------------|----------------------|
| 1   | Untreated.....                                         | 100                     | 100                    | 100                  |
| 2   | Manure extract.....                                    | 401                     | 255                    | 172                  |
| 3   | Ash of manure extract neutralized with $H_2SO_4$ ..... | 234                     | 137                    | 121                  |
| 4   | Ash of manure extract neutralized with $HNO_3$ .....   | 296                     | 195                    | 135                  |

From this table it will be seen at once that the relative order of growth in the different treatments is the same by all three criteria—transpiration, green weight, and dry weight. While the manure extract itself exerted a beneficial influence upon the plants, amounting to 301 per cent by transpiration, the ash of the same amount of extract when neutralized with sulphuric acid had a beneficial effect amounting to only 134 per cent by the same criterion. The ash which was neutralized with nitric acid showed an increase in growth intermediate between those of the other two treatments, this being 196 per cent by the criterion of transpiration. It is to be noted especially that in the extract of this soil the ash treated with nitric acid is not nearly as effective when compared with the untreated extract as is the same ash on the soil itself.

In removing the volatile and combustible portion of the manure extract there have been lost, besides the nitrates and nitrites, (1) all the organic matter originally present, and (2) all the ammonium compounds. When the ash is neutralized with nitric acid the amount of nitrates added is sufficient to replace not only the nitrates and nitrites originally present in the extract, but also to replace the organic acid radicals.

It therefore appears that by removing the ammonium compounds and organic matter and substituting nitric acid for the organic acids and nitrites of the original manure extract we have decreased the beneficial effect produced upon this soil extract from 391 to 196 per cent. It follows that the difference in amount between these two effects, 125 per cent, must be due either to the organic matter, to the ammonium compounds of the original manure extract, or to a combination of the two.

To gain evidence on the question as to how important ammonium compounds may be in this manure extract, an experiment similar to the last was carried out, but with addition of treatments which returned to the ash in the form of ammonium salts as much nitrogen as was contained in the original extract. A Kjeldahl determination of the total nitrogen content of the manure extract showed it to contain 2.66 grams of this element per liter. Ammonium nitrate and ammonium carbonate were used to replace this nitrogen which had been removed by burning. To the ash neutralized with sulphuric acid ammonium nitrate was added, and to that neutralized with nitric acid ammonium carbonate was added. In another culture ammonium nitrate equivalent to the nitrogen in the manure extract was added to the soil extract. The cultures treated with ash neutralized with nitric acid were accidentally injured, so that no results were obtained for this treatment. The data obtained are given in Table XXIX. The plants are shown in Plate II, fig. 2.

TABLE XXIX.—*Comparison of the effect of manure extract, ash from the same, and ash plus ammonium salts.*

[Plate II, fig. 2.]

| No. | Treatment of soil extract.                                                                                       | Relative transpiration. | Relative dry weight of tops. |
|-----|------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------------|
| 1   | Untreated.....                                                                                                   | 100                     | 100                          |
| 2   | Manure extract.....                                                                                              | 320                     | 165                          |
| 3   | Ash of above neutralized with $\text{H}_2\text{SO}_4$ .....                                                      | 154                     | 138                          |
| 4   | Same ash + $\text{NH}_4\text{NO}_3$ , to replace total N of original extract.....                                | 184                     | 157                          |
| 5   | Ash neutralized with $\text{HNO}_3$ + $(\text{NH}_4)_2\text{CO}_3$ , to replace total N of original extract..... | 230                     | 135                          |
| 6   | Soil extract + $\text{NH}_4\text{NO}_3$ equivalent to the N of manure extract.....                               | 160                     | 94                           |

Here the manure extract gave an increase of 220 per cent in transpiration and 65 per cent in dry weight, while the treatment with nitrate ash and ammonium carbonate gave only 130 per cent in transpiration and 35 per cent in dry weight. Owing to some undetermined cause, the dry weight of No. 3 is slightly greater than that of No. 5, although the difference by total transpiration is much greater and in the opposite direction. In this case the criterion of transpiration is doubtless more reliable than the other, as is indicated by Plate II, fig. 2. It appears that when the organic acid radicals and

that of nitrous acid are replaced by the nitric acid radical and when the ammonium compounds are considerably more than replaced by ammonium carbonate (all of the nitrogen of the manure extract besides the nitrates can not be in the form of ammonium salts, but some at least must exist in organic compounds) the effect produced is still much less than that produced by the original manure extract. It thus seems clear that the beneficial effects of the manure extract are due in part to the inorganic salts, but that they are undoubtedly due in part to organic materials.

In the soil extract it appears impossible to get the full effect from the manure extract by substituting nitric acid for the organic acids. An experiment was performed to determine the relative effectiveness of treatment of extract of Department soil with manure extract No. 3 and with sodium nitrate, this salt being added in an amount sufficient to contain a quantity of nitrogen equivalent to that added in the manure extract. This experiment comprised two bottles for each treatment, each bottle having ten plants. It lasted from November 24 to December 2. The results are given in Table XXX, and the plants are shown in Plate III, fig. 1.

TABLE XXX:—*Effect of manure extract and sodium nitrate on extract of Department soil.*

[Plate III, fig. 1.]

| No. | Treatment of soil extract.                                    | Relative transpiration. | Relative green weight of tops. | Relative dry weight of tops. |
|-----|---------------------------------------------------------------|-------------------------|--------------------------------|------------------------------|
| 1   | Untreated.....                                                | 100                     | 100                            | 100                          |
| 2   | Manure extract.....                                           | 400                     | 213                            | 151                          |
| 3   | NaNO <sub>3</sub> , to replace total N of manure extract..... | 196                     | 112                            | 109                          |

From these results it is clear that sodium nitrate in this concentration does not approach the manure extract in effectiveness. This strengthens the conclusion that the beneficial action of the latter can not be attributed mainly to the presence of nitrate therein.

The general conclusion which follows from the experiments on manure extract thus far given is that somewhat more than one-half of the beneficial effect of this substance is due to the nitrates and nonvolatile bases, that a small part is due to the ammonium base, and that somewhat less than a half is due to the direct action of organic substances.

Interesting information regarding the action of manure extract upon soil extract and upon nutrient solution was obtained by dialyzing the extract before using it. To hasten the process of dialysis, which is very slow as ordinarily used, and also to obtain a more thorough separation of the dialyzable from the nondialyzable portion of the manure extract, the material was placed between two cylinders

of parchment paper, the whole being immersed in a beaker of distilled water slightly larger than the larger parchment cylinder. The electric current from the lighting circuit was passed between two platinum electrodes, one being immersed in the inner parchment cylinder, and the other in the water contained in the beaker. The acids were obtained at one electrode and the bases at the other, while the nondialyzable materials remained between the two parchment cylinders. From time to time, as the process went on, the acid and alkaline solutions were removed and replaced by distilled water. The two solutions were mixed and preserved in a flask. After twenty-four hours of the action of the current it was found that practically all of the dialyzable materials originally present in the manure extract had been removed. The solution of these substances was always slightly acid, probably from loss of ammonia during the process of dialysis. It was neutralized before being used in the cultures, by the addition of a slight amount of potassium hydrate. During the process of dialysis a considerable portion of the nondialyzable materials were precipitated on the walls of the parchment paper tubes. A large portion, but not quite all, of the precipitate was redissolved upon diluting the mixture. The experiments were planned to test the relative effects produced by the dialyzable portions of manure extract No. 3 when added to extract of Department soil and to Knop's nutrient solution having a total content of 448 parts per million. At the same time nutrient solution and soil extract were treated with the original manure extract and with a solution formed by mixing the dialyzable and nondialyzable portions in equivalent amounts, thus reproducing the original extract as far as the amounts of the two kinds of material were concerned. Owing to the water added in the process of separation, it was impossible to add the reunited materials to the soil extract in the proportion of 1 to 20 as used in all other cases. The mixture was therefore added in the proportion of 1 to 40 in this case and a comparison culture was grown with the original manure extract added to soil extract in this proportion. By this procedure it were possible to compare the effect of the original manure extract with the reunited materials after dialysis.

The experiment just outlined was carried out three successive times, the results of the separate trials being in very close agreement. A single series of perfectly typical results is given in Table XXXI. Only the transpiration data were obtained. The plants are shown in Plate III, fig. 2, and Plate IV, fig. 1. Of course it is to be understood that placing the relative transpiration in this and the following table at 100 for the untreated soil extract and also for the untreated nutrient solution is only for convenience in comparing the untreated medium with its respective treatments; data from soil extract are not comparable with those from nutrient solution by this arrangement.

TABLE XXXI.—*Effect of dialyzable and nondialyzable portions of manure extract upon extract of Department soil and upon Knop's nutrient solution.*

[Plate III, fig. 2, and Plate IV, fig. 1.]

| Treatment.                                                         | Relative transpiration. |
|--------------------------------------------------------------------|-------------------------|
| Extract of Department soil.....                                    | 100                     |
| Same, + manure extract, $\frac{1}{20}$ .....                       | 342                     |
| Same, + dialyzable portion of manure extract.....                  | 220                     |
| Same, + nondialyzable portion of manure extract.....               | 121                     |
| Same, + manure extract, $\frac{1}{15}$ .....                       | 392                     |
| Same, + dialyzed and reunited manure extract, $\frac{1}{20}$ ..... | 199                     |
| Nutrient solution, 448 p. p. m. total salts.....                   | 100                     |
| Same, + manure extract, $\frac{1}{20}$ .....                       | 156                     |
| Same, + dialyzable portion of manure extract.....                  | 68                      |
| Same, + nondialyzable portion manure extract.....                  | 141                     |
| Same, + dialyzed and reunited manure extract.....                  | 86                      |

The dialyzable portion is more effective on the soil extract than is the nondialyzable portion, the latter having only a comparatively slight effect, while with the nutrient solution the dialyzable portion has a depressing effect and the nondialyzable portion has more than two-thirds as great an effect as has the original manure extract. The reunited manure extract is much less effective in both cases than the original, having a depressing action in the case of the nutrient solution. This latter fact indicates that the process of electrolytic dialysis has, in itself, decreased the effectiveness of the manure extract to accelerate growth. If the beneficial action of the manure extract were mainly due to the inorganic salts this would not be the case. Indeed, the dialyzed and reunited extracts contain a trace more potassium than the original, since the slight acidity resulting from dialysis was neutralized with potassium hydrate, and therefore, on the supposition made above, the reunited extract should be slightly more beneficial than the original. As to why the dialyzable portion should be more active on the soil extract, it may be suggested that the deleterious bodies of the soil extract may be best counteracted by the electrolytes, while the injurious materials which appear to emanate from the wheat roots may be best overcome by the nonelectrolytes. This would seem to be in disagreement with the conclusion reached from the experiments upon manure ash, but it is probable that the process of electrolytic dialysis has altered the substance of the manure extract very profoundly, even throwing some of the nondialyzable part out of solution, so that the former series of experiments is not directly comparable with the present one. On the whole, it appears that the evidence from these experiments on electrolytically dialyzed manure extract is decidedly in favor of the idea that the action of this fertilizer is in large part due to substances other than inorganic salts.

Another sample of the same manure extract was dialyzed by the ordinary method. The material was placed in a dialyzer tube sur-

rounded by distilled water and was allowed to remain, with frequent changes of the water, until practically all of the electrolytes had diffused out. This point was determined by taking the electrical resistance of the material from time to time. The process took several weeks, and fermentation, which had ceased in the original extract, may have been renewed with the disturbance of equilibrium which accompanied dialysis. The two portions were used in exactly the same way as in the case of the electrolytically dialyzed material.

The untreated manure extract and the reunited dialyzed extract were added to the soil extract and to the nutrient solution in the proportion of 1 to 19, and the dialyzable and nondialyzable portion of 1 part of manure extract were added to 19 parts of the soil extract and of the nutrient solution. This test was performed three times and, as the results were in close agreement, an average was made of the three different tests. The relative figures for these averages, according to transpiration, green and dry weight of tops, and dry weight of roots, are given in Table XXXII. The plants from one of the tests are shown in Plate IV, fig. 2, and Plate V, fig. 1.

TABLE XXXII.—*Effect of dialyzable and nondialyzable portions of manure extract on extract of Department soil and upon Knop's nutrient solution, averages from three experiments.*

[Plate IV, fig. 2, and Plate V, fig. 1.]

| Treatment.                                           | Relative transpiration. | Relative green weight of tops. | Relative dry weight of tops. | Relative dry weight of roots. |
|------------------------------------------------------|-------------------------|--------------------------------|------------------------------|-------------------------------|
| Extract of Department soil.....                      | 100                     | 100                            | 100                          | 100                           |
| Same, + manure extract.....                          | 264.3                   | 250.5                          | 148.9                        | 133.3                         |
| Same, + dialyzable portion of manure extract.....    | 263.3                   | 204.8                          | 124.2                        | 135.5                         |
| Same, + nondialyzable portion of manure extract..... | 199.4                   | 162.9                          | 109.0                        | 175.2                         |
| Same, + reunited manure extract.....                 | 269.8                   | 229.9                          | 132.2                        | 144.4                         |
| Nutrient solution in 224 p. p. m.....                | 100                     | 100                            | 100                          | 100                           |
| Same, + manure extract.....                          | 111.0                   | 148.8                          | 134.3                        | 92.2                          |
| Same, + dialyzable portion of manure extract.....    | 115.3                   | 153.0                          | 131.9                        | 92.0                          |
| Same, + nondialyzable portion of manure extract..... | 116.4                   | 121.6                          | 121.9                        | 96.6                          |
| Same, + reunited manure extract.....                 | 99.9                    | 154.2                          | 136.8                        | 95.3                          |

From these data, as well as from the illustrations, it is apparent that all treatments produced a gain in growth, excepting in the case of dry weight of roots in nutrient solution, which uniformly showed a loss. In all cases the reunited manure extract had practically the same effect as the untreated. This fact supports the idea suggested above that the failure to get this result with the electrically dialyzed material was due to some change in the chemical nature of the solutes, brought about by the electric process of dialysis. It is also apparent that both dialyzable and nondialyzable portions were markedly beneficial. Upon the soil extract the dialyzable portion produced a greater growth of tops than did the nondialyzable portion, while there was a difference in the opposite direction in the case of the

roots. Upon the nutrient solution the effect of the dialyzable portion equaled that of the nondialyzable portion, according to transpiration, but fell somewhat below it according to the other data, as well as according to the general appearance of the plants.

That the effects produced by the two portions of the manure extract used separately should be in general so nearly equal to those produced by the untreated extract is difficult to explain. The observation itself makes it clear that the increased growth due to manure extract is not primarily a phenomenon of simple nutrition; if it were, the nondialyzable portion should have little or no effect. It seems that in the natural manure extract there is more material than is necessary to bring about the increase of growth which follows its use. Yet a further dilution of the untreated extract failed to produce as good results and it is thus suggested that the dialyzable and nondialyzable portions may each interfere in some way with the action of the other.

What is most interesting in this connection is the fact that the nondialyzable portion of the naturally dialyzed manure extract had a marked beneficial effect. Since this portion contained practically nothing which can be considered as nutrient material for wheat plants, it follows that the organic matter which it contained acted indirectly to increase growth, probably, as has been suggested in preceding paragraphs, through some correcting influence which it brought to bear upon the toxic bodies of the soil extract and those which appear to be produced by the seedlings.

#### EXPERIMENTS WITH GREEN MANURE.

Several experiments were performed to determine the relative values of expressed juice of red clover (*Trifolium pratense*) and of the residue from pressing, consisting largely of insoluble matter, especially cellulose. One thousand grams of the ground clover leaves which were used were found to contain 126 grams of dry residue, while the expressed juice contained 57.4 grams of dry matter. The results of a typical experiment on comparative effects of ground clover leaves and of the residue, juice, and dried juice from the same, upon Takoma lawn soil, are given in Table XXXIII. The ground leaves were used in the proportion of 5,000 parts, computed on dry matter, per million parts of soil. The cultures with the residue and those with the juice, contained in the same amount of soil an amount of these substances equivalent to the amount found in the quantity of ground leaves used in the first culture. Thus the results furnish data on the relative value of these materials when applied to this soil.

TABLE XXXIII.—*Effects of clover leaves, clover juice, etc., upon Takoma soil.*

[P. p. m. = parts per million.]

| Treatment of soil.                       | Relative transpiration. | Relative green weight. |
|------------------------------------------|-------------------------|------------------------|
| Untreated soil.....                      | 100                     | 100                    |
| Ground clover leaves, 5,000 p. p. m..... | 165                     | 161                    |
| Juice from same.....                     | 154                     | 160                    |
| Juice from same, dried.....              | 132                     | 143                    |
| Residue from same.....                   | 114                     | 108                    |

It appears that the expressed juice from the clover contains practically all the beneficial substances. The effect of the residue is probably due to the small amounts of juice which the repeated washing and pressing had failed to remove. In preparing this soil for the basket cultures the physical condition is usually brought to its optimum, so that the purely physical effect of adding such fibrous material as the pressed residue should be practically negligible. Drying the juice appears to have reduced its efficiency about one-half. Similar results were obtained from other experiments.

It appears, therefore, that aside from the purely physical effect of incorporating fibrous material into the soil—an effect which is not likely to be manifest in the wire-pot cultures, because of the excellent physical condition of the potted soil—the valuable portion of clover as a fertilizer lies in its juice.

The question at once arises; as in the case of manure extract above dealt with, whether the beneficial portion of the expressed juice is organic or inorganic in its nature. To gain some evidence upon this question a comparison was made, by the method of bottle cultures, of the effect of the addition of dried clover juice and of a corresponding amount of nutrient salts to the aqueous extract of Takoma soil. It was found that the dried juice contained 10 per cent of ash. Accordingly the experiment comprised an addition to the soil extract of 100 and 200 parts per million of this material and of 10 and 20 parts per million of nutrient salts, the salts used being calcium nitrate, potassium nitrate, magnesium sulphate, and potassium acid phosphate in the ratios of 4 : 1 : 1 : 1, by chemical equivalents. The results are given in Table XXXIV. Only the criterion of transpiration was used for comparing the growth of the plants.

TABLE XXXIV.—*Effects of dried clover juice and of nutrient salts on extract of Takoma soil.*

[P. p. m.=parts per million.]

| Treatment.                           | Relative transpiration. |
|--------------------------------------|-------------------------|
| Untreated soil.....                  | 100                     |
| Dried clover juice, 100 p. p. m..... | 198                     |
| Nutrient salts, 10 p. p. m.....      | 111                     |
| Dried clover juice, 200 p. p. m..... | 328                     |
| Nutrient salts, 20 p. p. m.....      | 145                     |

These results seem to indicate that by far the most important factor in the effectiveness of the dried juice was the organic matter. The salts added were of the form and in the proportions to make an excellent nutrient solution, very similar to the one in common use, which is known as Knop's solution.<sup>a</sup>

#### SUMMARY.

These experiments indicate that the beneficial effects derived from the use of stable manure and green manure, at least upon Takoma lawn soil and Department soil, are probably, in large part, due to the organic matters therein contained rather than to the salts carried into the soil by such treatments. That the organic matter is directly of use to plants as nutrient material is not probable; it appears to be beneficial largely through some action upon the soil constituents. As has been shown above in this bulletin, as well as in Bulletin 28 of the Bureau of Soils, the soils here experimented upon contain bodies which are distinctly toxic to wheat plants, and it may be that the organic materials of these manures are effective in so altering the toxic substances as to render them harmless.

#### THE PREPARATION OF NONTOXIC DISTILLED WATER.

##### LITERATURE.

It is well known to both plant and animal physiologists that distilled water which is considered pure enough for ordinary chemical operations is usually totally inadequate for physiological experimentation. As a nontoxic water was absolutely necessary for the investigations in soil fertility reported in this bulletin, an investigation of methods by which physiologically harmless water may be procured was undertaken. Since the literature of this subject is considerably scattered, a brief review will not be out of place. The statement that distilled water is per se injurious to the lowest forms of organisms appears a number of times in the earlier papers on chemical stimulation. In some of these earlier contributions the apparent ill effects of distilled water are ascribed to the absence of certain salts rather than to a deleterious action of the water itself. In 1891 Loew,<sup>b</sup> in answering Aschoff's statement<sup>c</sup> that distilled water is toxic, calls attention to the now famous work of Nägeli, at that time unpublished, upon the effect of copper and other metals on plants, with the sugges-

<sup>a</sup> The modification of Knop's solution here used has been described by Livingston, *Chemical Stimulation of a Green Alga*, *Bul. Torr. Bot. Club*, **32**, 1-34 (1905).

<sup>b</sup> Loew, O., *Bemerkungen über die Giftwirkung des destillirten Wassers*, *Landwirthsch. Jahrb.*, **20**, 235 (1891).

<sup>c</sup> Aschoff, C., *Über die Bedeutung des Chlors in der Pflanze*, *Landwirthsch. Jahrb.*, **19**, 113-141 (1890).

tion that Aschoff's results were probably to be explained on the ground that the water used was impure. On the following page of the same journal appeared a note by Schulze<sup>a</sup> to the effect that Frank's statement<sup>b</sup> that lupine seedlings are poisoned by distilled water is probably based upon work done with impure water.

The work of Carl von Nägeli,<sup>c</sup> just referred to, did not appear until 1893, being a posthumous paper and more than ten years old at the time of its publication. It is accompanied by an introduction by S. Schwendener and a final chapter by C. Cramer. In perusing this paper the reader follows the series of logical steps by which the author gradually approached the truth in regard to the toxicity of waters. In 1880 Nägeli made the discovery that the death phenomena of *Spirogyra*, when the plant is killed by a rather strong solution of silver nitrate, are very different from those observed when death is brought about by means of a very weak solution of the same salt. He termed the former chemical, and the latter olygodynamic death. Cramer was able to substantiate these observations. In the light of more modern work it appears possible that this difference between death by a strong and by a weak solution may be related to the commonly observed chemical acceleration of growth, etc., by weak solutions of substances which kill in higher concentration. With rather strong solutions of copper, one form of death ensues; with weaker solutions, another form of death ensues, and with still weaker solutions it is probable that growth would be accelerated, although we are not aware that this has been shown for *Spirogyra*.<sup>d</sup> Nägeli found olygodynamic death to occur when small amounts of *Spirogyra* were placed in relatively large amounts of ordinary distilled water or of tap water which had stood for some time in contact with lead pipes and brass faucet. It was shown that this is due to the extremely small amounts of copper and other metals dissolved by the water. Similar effects were obtained from tin, iron, and mercury, but copper was by far the most toxic.

Nägeli also found that when water which had been made toxic by standing in contact with copper was allowed to stand in glass or platinum vessels, these vessels, after thorough washing, retained sufficient

<sup>a</sup> Schulze, E., Über das Verhalten der Lupinenkeimlinge gegen destillirtes Wasser, Landwirthsch. Jahrb., **20**, 236 (1891).

<sup>b</sup> Frank, B., Untersuchungen über die Ernährung der Pflanze mit Stickstoff, und über den Kreislauf desselben in der Landwirtschaft, Landwirthsch. Jahrb., **17**, 421-553 (1888).

<sup>c</sup> Nägeli, C. von, Über olygodynamische Erscheinungen in lebenden Zellen, Neue Denkschr. schweiz. Gesell. Naturforsch. (Nouv. Mém. de la Soc. Helvétique des Sci. Nat.), **33**, Abth. I, the second contribution, paged 1-52 (1893).

<sup>d</sup> For some remarks on the relation of the acceleration of life phenomena to death, see Livingston, B. E., Chemical Stimulation of a Green Alga, Bul. Torr. Bot. Club, **32**, 1-34 (1905).

of the toxic metal to produce contamination of nontoxic water which was afterwards placed in them. Boiling the vessels in water for a long time resulted in the removal of most of this retained copper, but the best way to remove it was found to be by thorough washing in nitric and sulphuric acid, followed by water. This behavior is probably due to the adsorption of the copper on the surface of the glass. A number of other solids appeared to remove copper by adsorption from water contaminated with this metal. When copper-treated water was shaken with sulphur, graphite, soot, coal dust, peat, starch, Swedish filter paper, cotton, silk, wool, stearic acid, paraffin, feathers, etc., it was found to be greatly improved or entirely harmless. Cramer found ferric hydrate and artificially prepared iron rust to have the same effect. Nägeli explained this action of solids on the ground that the particles of copper cling to the solids and are thus removed from the water. He also found that colloidal solutions, such as gum, dextrin, and egg albumen destroyed the toxicity of copper-treated water, and explained this by supposing the copper to be taken up on the surfaces of the micellæ, of which his now famous micellar theory supposed these bodies to be composed. When large numbers of *Spirogyra* filaments were placed in toxic water the injurious effect failed to appear. This observation was explained by the supposition that all of the copper present was removed from the water by the filaments and that the amount thus taken up by any individual was below the toxic limit.

Concentration and analysis showed the distilled water used by this author to contain copper, lead, zinc, and iron. Redistilling in glass made this water harmless, indicating that the original toxic action was due to the presence of the metals, mainly copper. Throwing away the first portion of the distillate resulted in still better water. The toxicity of the distilled water used by Nägeli varied from time to time, this variation being probably due to variations in the amount of copper it contained.

The toxicity of distilled water to animals was studied by Ringer and his coworkers from 1883 to 1894, with the conclusion that the water itself was injurious. After the appearance of Nägeli's paper, Locke<sup>a</sup> conceived the idea that the toxicity observed by Ringer was probably due to impurities in the water. He was able to show that this is indeed true and that tadpoles and the fresh-water *Tubifex rivulorum*, while they die in ordinary distilled water live well in water redistilled and preserved in glass. Ringer<sup>b</sup> then repeated some of his former experiments and came to the same conclusion as that reached by Locke.

<sup>a</sup> Locke, F. S., On a Supposed Action of Distilled Water as Such on Certain Animal Organisms, Jour. Physiol., 18, 319-329 (1895).

<sup>b</sup> Ringer, Sydney, The Action of Distilled Water on Tubifex, Jour. Physiol., 22, Proc. XIV (1897).

In Ringer's work it was shown that the toxicity of distilled water was suppressed by the addition of several salts, especially those of calcium. After Locke and Ringer had shown that the toxicity of the water was due to impurities, especially copper, Miss A. Moore<sup>a</sup> called attention to the fact that the beneficial effects produced by salts must be considered as merely antagonizing the effects of the copper present.

Dehérain and Demoussy<sup>b</sup> published on the same question in 1901, controverting the conclusions of Boehm<sup>c</sup> that the failure of lupine seeds to germinate in distilled water was due to the absence of calcium, and showing that the observed phenomena were probably due to the use of toxic water. These authors show that seeds of white and yellow lupine germinate well in water redistilled in glass, but not in ordinary distilled water. When redistilled water stood in contact with copper the water became toxic. They also noted that wheat seedlings will not form the normal long roots of water cultures in copper-treated water, but that their development is normal in redistilled water. The toxic action of ordinary distilled water was markedly manifest in the case of wheat, even when it was diluted to twenty times its original volume with nontoxic redistilled water. The white lupine was injured in toxic water diluted in the same way to five times its original volume. Dehérain and Demoussy estimated the copper present in their ordinary distilled water to be from 0.1 to 0.2 milligram per liter. They observed that with a large number of lupine seedlings a given volume of toxic water failed to be injurious, but that with only a few seedlings the toxic action was well marked, and explained this fact in the same way as Nägeli explained his similar observation in the case of *Spirogyra*.

In 1904 Lyon<sup>d</sup> published the results of a study of the toxicity of distilled water, in which he used the relative development of sea-urchin (*Arbacia*) eggs as a criterion for comparing the different waters. Sea water was concentrated to a fraction of its original volume by boiling, and was then rediluted to its original volume by means of the water to be tested. This author came to the following conclusions: (1) The tap water at Woods Hole, Mass. (where the work was done) was decidedly toxic. It was much improved by boiling away one-third of its volume. (2) Automatic stills, when

<sup>a</sup> Moore, Anne, Further Evidence of the Poisonous Effects of a Pure NaCl Solution, *Am. Jour. Physiol.*, **4**, 386-395 (1900).

<sup>b</sup> Dehérain, P. P., and Demoussy, E., Sur la germination dans l'eau distillée, *Compt. rend.*, **132**, 523-527 (1901).

<sup>c</sup> Boehm, J., De l'influence des sels de chaux sur le développement des végétaux, *Annales agronom.*, **1**, 470-478 (1875). Also Über den vegetabilischen Nährwerth der Kalk salze, *Sitzungsber. d. K. Akad. d. Wissensch. Wien, Math.-Nat. Classe*, **71**, 287-304 (1875).

<sup>d</sup> Lyon, E. P., A Biological Examination of Distilled Water, *Biol. Bul.*, **6**, 198-202 (1904).

having a continuous influx of water to the retort, gave toxic water. (3) Even when the water was distilled from glass into glass, the first, second, and third tenths of the distillate exhibited toxic properties. The fourth tenth was of good quality. (4) The best water was obtained by distilling in glass, with potassium dichromate and sulphuric acid added to the retort. (5) Automatic stills gave a good water when sulphuric acid was added to the retort at the time the still was started. The water thus obtained was of better quality than that redistilled from glass without addition of acid to the retort, excepting where a large amount of the distillate was discarded at first.

The contributions cited previous to that of Lyon have agreed in ascribing the toxic properties of ordinary distilled water to the presence of nonvolatile poisons, mainly copper. It will be noticed at once that the toxic bodies dealt with by Lyon are, in part at least, volatile with steam, so that they appear in the distillate from glass, but that they are rendered nontoxic or nonvolatile by the action of chromic, and to some extent also by sulphuric acid. Lyon obtained evidence not only that the tap water at Woods Hole is toxic because of a volatile material, but that natural sea water is also similarly toxic to an appreciable degree. He suggests that the toxic properties dealt with by him may be due to the presence of traces of ammonia.

Ballot has recently published a paper on the toxicity of distilled water for the fresh-water *Gammarus*.<sup>a</sup> He shows that water redistilled in glass, quartz, and platinum are all quite toxic to this animal, although not as toxic as water merely distilled in the ordinary way from a copper boiler. This toxicity is counteracted by the addition of sodium chloride to the water, but the other salts present in the original tap water failed to produce any beneficial effect. The author concludes that the sodium chloride is beneficial in redistilled water because it is necessary for life, and that it is beneficial in ordinary distilled water both for this reason and because it counteracts the toxic effects of impurities.

Several methods for obtaining water of low electrical conductivity have been devised by physicists and chemists. It appears that carbon dioxide is the most important solute in raising the conductivity of distilled water, but that this is not the only substance ordinarily present. For protecting water from contamination by carbon dioxide it may be kept in tightly stoppered bottles or in containers connected with the external air only through a chamber containing an absorber for carbon dioxide.<sup>b</sup> A method which results in a water of low con-

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<sup>a</sup>Ballot, G., On the Toxicity of Distilled Water for the Fresh-water *Gammarus*. Suppression of this toxicity by the addition of small quantities of sodium chloride. Univ. of Calif. Publs. Physiology, **1**, 199-217 (1904).

<sup>b</sup>See Kohlrausch, F., Über Wasser in einigen Beziehungen zur Luft, Zeitschr. physik. Chem., **42**, 193-201 (1902).

ductivity, and one which may also be free from toxic properties, has been recently described by Bousfield.<sup>a</sup>

About a year ago it was observed in this laboratory that if the ordinary distilled water here used is shaken with purified carbon black or precipitated ferric hydrate and the solid is afterwards filtered out, the filtrate exhibits no appreciable toxicity to seedlings of wheat. This is in agreement with the results obtained by Nägeli with graphite, soot, and coal dust, and with that obtained by Cramer with ferric oxide. The explanation is doubtless that given by Nägeli that the solids remove the toxic substances from solution. Evidence that these solids, as well as several others, remove injurious bodies from the water in which they are dissolved has been furnished by True and Oglevee<sup>b</sup> and by Breazeale.<sup>c</sup>

True and Oglevee showed that the presence of sand, filter paper, paraffin, or potato starch in solutions of copper sulphate in which were growing seedlings of *Lupinus albus* is effective in decreasing to a marked degree the toxicity of the solution for these seedlings. A killing concentration may thus be reduced to a stimulating concentration or to one without physiological response, according to the amount of the solid present. They follow Nägeli in supposing that the solid removes the salt from solution and thus produces an effect closely similar to that of simple dilution. These authors were unable to get a similar reduction in the toxicity of solutions of phenol and resorcinol by the use of solids. They suggest that this may be due to the fact that these substances, in order to be injurious, need to be in more concentrated solution than does the copper salt, while the relative amount of dissolved substance removed from solution by the solids is, of course, much smaller in a more concentrated solution than in a weaker one.

Breazeale, working with wheat seedlings, found that carbon black acted in the case of copper sulphate in the same way as did the solid used by the authors just mentioned, but in the case of sulphuric acid he was unable to obtain any reduction in toxicity by the use of this solid.

#### EXPERIMENTATION.

As already mentioned, the distilled water of these laboratories is generally toxic to wheat seedlings. It is distilled from a copper boiler, condensed in a block-tin worm, collected and stored in a tin-lined cop-

<sup>a</sup> Bousfield, W. R., The Purification of Water by Continuous Fractional Distillation, Jour. Chem. Soc. (London), **87**, 740-747 (1905).

<sup>b</sup> True, R. H., and Oglevee, C. S., The Effect of the Presence of Insoluble Substances on the Toxic Action of Poisons, Bot. Gaz., **39**, 1-21 (1905). A preliminary report appeared in Science, N. S., **19**, 421-424 (1904). In this connection see also Dandeno, J. B., The Relation of Mass Action and Physical Affinity to Toxicity, etc., Am. Jour. Sci., **17**, 437-458 (1904).

<sup>c</sup> Breazeale, J. F., Effect of Certain Solids upon the Growth of Wheat in Water Cultures, Bot. Gaz., **41**, 54-63 (1906).

per tank, and conducted to the various rooms in block-tin pipes furnished with brass cocks. This treatment will doubtless be found to accord well with that prevailing in most well-equipped chemical laboratories. This water was studied from the standpoint of the toxicity problem by the use of wheat seedlings grown in water.

In making a study of the properties of the ordinary distilled water, with intent to determine something in regard to the probable source of the observed toxic action, the following results were obtained: In the first place, the most toxic water was that which had been standing for several days in the pipe. This had been in contact with block tin and probably with solder at several points, as well as with the brass of the stopcock. Water taken directly from the storage tank was not nearly so toxic. Still less toxic was that taken directly from the condenser. The latter varied in its properties from time to time, but it was impossible to relate these variations to the amount of water in the boiler (the still is not automatic and has no constant level device) or to the length of time the apparatus had been working. The water from the tank also varied considerably in its toxicity, being obviously worse when the still had not been in use for a day or two.

In order to get water as pure as was possible by redistillation, a double still was constructed of two glass retorts and a platinum condensing tube. Ordinary distilled water (from the storage tank) was placed in a retort, with addition of potassium dichromate and sulphuric acid. The steam from this was allowed to bubble through an alkaline solution of potassium permanganate in a second retort, which was kept heated to prevent the accumulation of the water by condensation. The steam from this second retort was conducted through the platinum condenser, which was so connected that no liquid water could enter it from the glass. The distillate was collected in a "Non-sol-glass" flask and stored in glass-stoppered bottles (Winchester acid bottles) composed of a greenish glass, which has been found to be extremely insoluble.

Several other stills consisting of a glass retort and a straight glass condenser were used for a single distillation, collecting the distillate in glass. The stills were provided with the usual bends or bulbs to prevent spray from reaching the condensers. Cork stoppers formed the necessary connections, and either Jena or "Non-sol-glass" flasks were always used.

The carbon black used in these studies was the "G Elf" brand, obtainable from dealers in painters' supplies. It appears to be prepared by burning petroleum or natural gas and collecting the product on cooled surfaces. Before using it in experiments it was thoroughly washed, first in ordinary distilled water and then in water redistilled in glass until there was practically no increase in conductivity of the supernatant water after being in contact with the carbon black for twenty-four hours.

The ferric hydrate used in most of these tests was precipitated from ferric nitrate with ammonia, and was washed in the same manner as that described for carbon black. Some ferric hydrate for a comparison, was prepared from ferric chloride and sodium hydrate in a similar way. The aluminum hydrate was prepared from aluminum sulphate and ammonia water in a manner similar to that used for the ferric hydrate. The quartz flour was a good quality of pulverized French flint. It was not washed, and contained traces of soluble material.

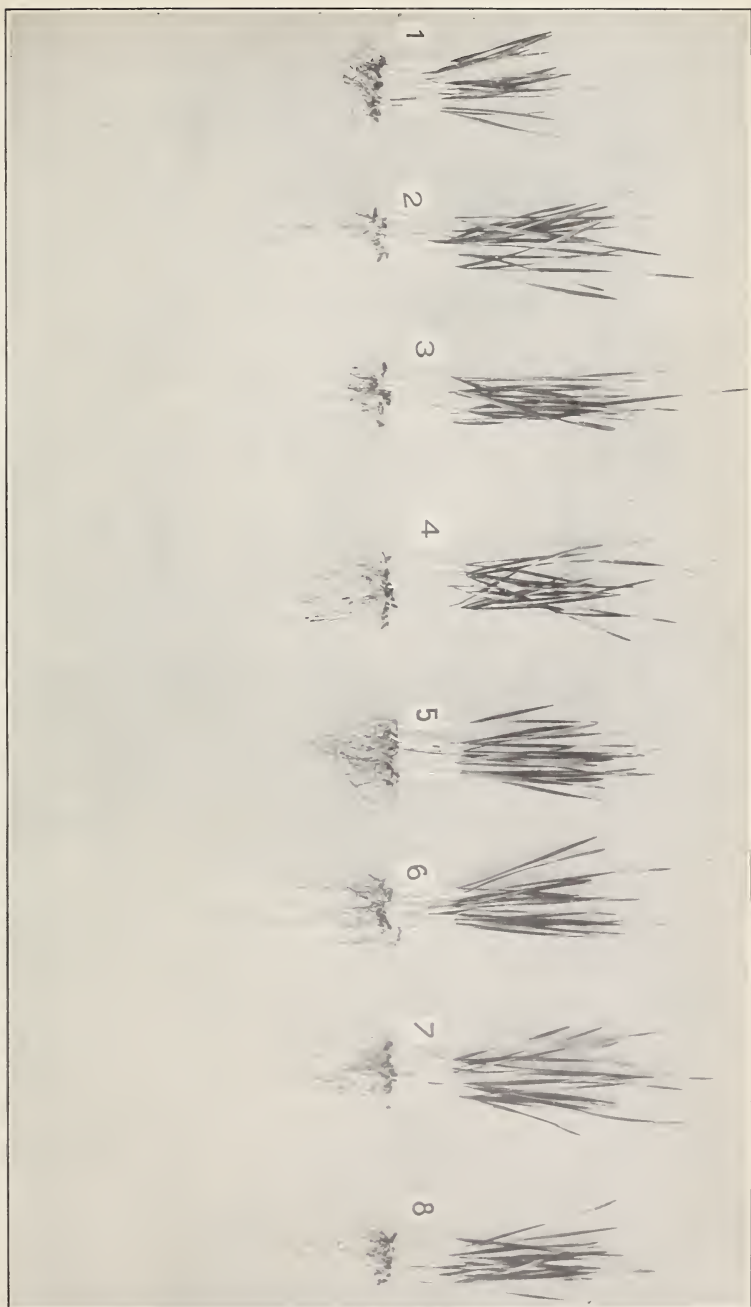
The carbon black, ferric hydrate, and aluminum hydrate were used in the moist condition, the amount of dry material used in each treatment of 50 c. c. of solution being about 0.75 gram of carbon black, 0.63 gram of ferric hydrate, and 0.40 gram of aluminum hydrate. The quartz flour was used in the proportion of 25 grams per liter.

A comparison of the effects produced by several different waters upon wheat cultures is shown by the following experiment. In this series of cultures water redistilled in the double still with platinum condenser was compared with water redistilled in glass with glass condenser without addition of anything to the retort. It was also compared with ordinary distilled water from the storage tank and with ordinary distilled water which had stood in the supply pipe for two days. The data of total transpiration and of green weight of tops are given in Table XXXV. For ease in comparison the observed quantities are reduced to a uniform basis of 100 for the first redistilled water.

TABLE XXXV.—*Comparison of the effect of different distilled waters.*

| Description of water.                                                                  | Relative transpiration. | Relative green weight. |
|----------------------------------------------------------------------------------------|-------------------------|------------------------|
| Redistilled from $K_2Cr_2O_7 + H_2SO_4$ and from $KMnO_4$ with platinum condenser..... | 100                     | 100                    |
| Redistilled water, glass condenser.....                                                | 110                     | 114                    |
| Ordinary distilled (tank).....                                                         | 67                      | 83                     |
| Ordinary distilled (stood in pipe).....                                                | 58                      | 100                    |

These figures show that by both criteria—transpiration and green weight—the water redistilled in glass without the addition of chemical reagents to the retort, is the best, while that redistilled in the compound still is better than either sample of ordinary distilled water. In this experiment the water from the supply tank appears somewhat better than that from the pipe, according to the criterion of transpiration, but the reverse is true of these two samples by the criterion of green weight, although in other experiments the tank water was better by both transpiration and green weight than water which had stood in the pipe. Water taken directly from the main laboratory supply varies, as has been stated. The tests showed it to be sometimes as good as or even slightly better than water redistilled in the double still, and at other times it was markedly inferior to this.



WHEAT SEEDLINGS GROWN IN DISTILLED WATER TREATED WITH FERRIC HYDRATE, ALUMINUM HYDRATE, AND CARBON BLACK.

[1. Ordinary distilled water. 2. Ordinary distilled water treated with ferric hydrate. 3. Ordinary distilled water treated with aluminum hydrate. 4. Ordinary distilled water treated with carbon black. 5. Redistilled water untreated. 6. Redistilled water treated with ferric hydrate. 7. Redistilled water treated with aluminum hydrate. 8. Redistilled water treated with carbon black.]



From these results it appears that the toxicity of the ordinary distilled water of these laboratories is at least in part due to a deleterious action of the tank and supply pipes and that it is partially corrected by redistilling. It is not, however, entirely corrected, as was shown by comparing the effects produced by water obtained by redistilling in glass with a glass condenser when several substances were added to the retort, with the effects produced by simply redistilling. The effect of adding sulphuric acid, potassium dichromate, and both of these together, is shown in the two experiments presented in Tables XXXVI and XXXVII. A test of the toxicity of the water remaining in the retort was included in the first experiment. The relative results of transpiration, green and dry weights of tops, and dry weight of roots, are given in the tables. It will be noted that in the first table ordinary distilled water is stated on the basis of 100. In Table XXXVII redistilled water is stated on the basis of 100, and the electrical conductivities of the waters are also given.

TABLE XXXVI.—*Comparison of the effect of different waters.*

| Description of water.                                                            | Relative transpiration. | Relative green weight of tops. | Relative dry weight of tops. | Relative dry weight of roots. |
|----------------------------------------------------------------------------------|-------------------------|--------------------------------|------------------------------|-------------------------------|
| Ordinary distilled.....                                                          | 100                     | 100                            | 100                          | 100                           |
| Redistilled.....                                                                 | 171                     | 120                            | 124                          | 136                           |
| Undistilled portion from above.....                                              | 136                     | 118                            | 95                           | 100                           |
| Redistilled with $\text{H}_2\text{SO}_4 + \text{K}_2\text{Cr}_2\text{O}_7$ ..... | 229                     | 150                            | 162                          | 171                           |

TABLE XXXVII.—*Effect of adding to the retort sulphuric acid, potassium dichromate, and both together, in redistilling water in glass with glass condenser.*

| Description of water.                                                                      | Relative transpiration. | Specific electrical conductivity. |
|--------------------------------------------------------------------------------------------|-------------------------|-----------------------------------|
| Redistilled.....                                                                           | 100                     | $3.68 \times 10^{-6}$             |
| Same, + $\text{H}_2\text{SO}_4$ added to retort.....                                       | 175                     | $2.31 \times 10^{-6}$             |
| Same, + $\text{K}_2\text{Cr}_2\text{O}_7$ added to retort.....                             | 135                     | $2.20 \times 10^{-6}$             |
| Same, + $\text{H}_2\text{SO}_4$ and $\text{K}_2\text{Cr}_2\text{O}_7$ added to retort..... | 123                     | $2.36 \times 10^{-6}$             |

It is evident from all three criteria that a much better water was obtained by the addition of one or both of these substances to the retort than that prepared by simple redistillation. From Table XXXVII it seems that the addition of sulphuric acid alone is considerably better than any of the other treatments. Other experiments pointed to the same result, though the difference between the results obtained by the two single additions and by the double one were not always as marked as here. The water redistilled without reagents has a much higher conductivity than the others, and this suggests that the toxic bodies present in the former may be, in part, at least, electrolytes. They are, of course, volatile with steam in the absence of reagents. for the differences observed by the different methods of redistilling

would not otherwise be manifest. This point is emphasized by the fact that the residue from redistillation (Table XXXVI) is much improved according to transpiration and green weight, and little if at all injured according to the other two criteria. Sulphuric or chromic acid appears to decompose or fix those injurious substances present in ordinary distilled water which are originally volatile, so that they are made either nonvolatile or nontoxic, and hence they largely fail to appear in the distillate. Further evidence that a portion of the toxic material of the ordinary distilled water is volatile with steam was obtained by merely boiling for five minutes. This treatment often produced a better water than was obtained by redistillation. One of these experiments gave a gain for the culture in boiled water of 36 per cent in transpiration above that in water redistilled in glass. The plants of this experiment are shown in Plate V, figure 2, which brings out the difference in growth of tops and roots even more plainly than do the transpiration figures. The fact that Table XXXVI shows an improvement in the residue from redistillation as well as in the distillate can be explained by assuming that a portion of the toxic bodies of the original water had escaped from the apparatus or had been altered by the heat so as to be less harmful.

One observation on the physiological effects produced by the volatile and nonvolatile impurities of this distilled water may be noted here, namely, that in ordinary distilled water the growth of wheat seedlings for the first ten days is practically the same whether or not the endosperm is removed, while in water which has been simply redistilled (and has thus been freed from the nonvolatile injurious substances but not from the volatile ones) the difference between plants with and without endosperm is normal. The plants without endosperm in redistilled water give transpiration figures more than twice as great as do those with or without endosperm in ordinary distilled water. Uninjured seedlings in redistilled water gave a total transpiration about 30 per cent greater than that obtained in the same water from seedlings with the endosperm removed. This may mean that one of the first effects of the ordinary distilled water is to hinder the digestion and use of the materials stored in the endosperm.

In considering the probable sources of the nonvolatile portion of the toxic materials present in the ordinary distilled water of these laboratories the effect of metallic tin was tested. A piece of block tin tubing, similar to that used in piping the distilled water about the building, was split, hammered into a flat plate, and filed bright. This was cut into several pieces, altogether having a surface of about 100 sq. cm., and the pieces were allowed to stand in contact with 500 c. c. of simple redistilled water for two days, after which the toxicity of the water so treated was compared with the original untreated water by

means of the usual wheat cultures. The results showed the two waters to be identical; the tin had exerted no ill effect. Water was again placed upon the tin and the experiment was repeated after it had stood in contact for five and one-half months, this time with quite a different result. The total transpiration for the culture in untreated redistilled water was 23 grams, while for the other it was but 15.5 grams. It thus appears that the greater toxicity usually observed in case of distilled water which had stood only a few days in the black tin pipes is not due to the tin, but that the tin lining of the storage tank may furnish some of the injurious material which is exhibited in water from the receptacle owing to the greater period of contact. It is probable, however, that most of the metallic poison of the ordinary distilled water is copper, coming perhaps in part from the imperfections in the tin lining of the tank, and, in the case of the water which has stood in the pipes, from the brass of the cocks. No experiments were made with copper, its solubility and toxicity having been well established by Nägeli and Cramer, already cited, as well as by other workers, and by the recent work by Moore and Kellerman<sup>a</sup> on the sensitiveness of plant organisms to copper poisoning.

The effect of the finely divided solids was studied on ordinary distilled water and on redistilled water condensed in platinum and in glass. This water, which has been subjected to the absorbing action of these solids, but which contains none of the solid at the time it is used in the cultures, will be termed treated water.

Three typical experiments are represented in Tables XXXVIII to XL and in Plates VI and VII, showing the effects of finely divided solids on distilled waters. All data are calculated to a uniform basis of 100 for ordinary distilled water.

TABLE XXXVIII.—*Effect of carbon black, ferric hydrate, and quartz flour treatments upon ordinary distilled water and upon water redistilled from chromic acid and from potassium permanganate and condensed in platinum condenser.*

| Treatment.          | Ordinary distilled water. |                        |                      | Redistilled water.      |                        |                      |
|---------------------|---------------------------|------------------------|----------------------|-------------------------|------------------------|----------------------|
|                     | Relative transpiration.   | Relative green weight. | Relative dry weight. | Relative transpiration. | Relative green weight. | Relative dry weight. |
| Untreated.....      | 100                       | 100                    | 100                  | 219                     | 129                    | 120                  |
| Carbon black.....   | 209                       | 121                    | 118                  | 276                     | 94                     | 92                   |
| Ferric hydrate..... | 228                       | 114                    | 102                  | 221                     | 118                    | 104                  |
| Quartz flour.....   | 245                       | 137                    | 114                  | 233                     | 122                    | 108                  |

In the experiment presented in Table XXXVIII the increase obtained by redistilling was 119 per cent by transpiration. Treatment by means of the solids brought the ordinary water to about the

<sup>a</sup> Moore, G. T., and Kellerman, K. F., A Method of Destroying or Preventing the Growth of Algae and Certain Pathogenic Bacteria in Water Supplies, Bul. 64, Bureau of Plant Industry, U. S. Dept. of Agr. (1904).

Moore, G. T., and Kellerman, K. F., Copper as an Algicide and Disinfectant in Water Supplies, Bul. 76, Bureau of Plant Industry, U. S. Dept. of Agr. (1905).

same productiveness as was exhibited by the redistilled, and treatment of the redistilled by means of the solids gave a somewhat better growth, although this is not very great, and fails to be shown by green and dry weights in the case of carbon black.

TABLE XXXIX.—*Same distilled waters and same treatments as in Table XXXVIII.*

[Plate VI.]

| Treatment.          | Ordinary distilled water. |                        | Redistilled water.      |                        |
|---------------------|---------------------------|------------------------|-------------------------|------------------------|
|                     | Relative transpiration.   | Relative green weight. | Relative transpiration. | Relative green weight. |
| Untreated.....      | 100                       | 100                    | 156                     | 115                    |
| Carbon black.....   | 398                       | 127                    | 274                     | 110                    |
| Ferric hydrate..... | 368                       | 120                    | 430                     | 151                    |
| Quartz flour.....   | 393                       | 124                    | 366                     | 137                    |

In the experiment presented in Table XXXIX simple redistillation showed an increase in transpiration of 56 per cent, while the increase produced by treatment of the ordinary water with the solids was from 268 to 298 per cent. Similar treatment of the redistilled water, while it gave marked increases over redistillation alone, failed to give any increase over the treated original water excepting in the case of ferric hydrate, which increased transpiration considerably. The plants of this experiment are shown in Plate VI.

TABLE XL.—*Effect of carbon black, ferric hydrate, and aluminum hydrate treatments upon ordinary distilled water and upon water redistilled in glass.*

[Plate VII.]

| Treatment.            | Ordinary distilled water. |                        |                      | Redistilled water.      |                        |                      |
|-----------------------|---------------------------|------------------------|----------------------|-------------------------|------------------------|----------------------|
|                       | Relative transpiration.   | Relative green weight. | Relative dry weight. | Relative transpiration. | Relative green weight. | Relative dry weight. |
| Untreated.....        | 100                       | 100                    | 100                  | 120                     | 106                    | 108                  |
| Carbon black.....     | 210                       | 106                    | 101                  | 151                     | 106                    | 104                  |
| Ferric hydrate.....   | 257                       | 109                    | 104                  | 182                     | 120                    | 110                  |
| Aluminum hydrate..... | 210                       | 108                    | 102                  | 182                     | 101                    | 104                  |

The experiments of Table XL (see also Plate VII) showed increases in transpiration of 20 per cent due to redistilling, of from 110 to 157 per cent due to treatment of original water with solids, and of 51 to 82 per cent due to treatment of the redistilled water with solids. Aluminum hydrate is seen to have practically the same effect as carbon black and ferric hydrate.

Summarizing the results given in the preceding tables, together with those of many other similar tests, it appears that treatment of the ordinary water by means of the solids always produces a very excellent water. Redistilling by both of the two methods used appears to have the same effect and to improve the water to a marked degree, but this treatment, save in exceptional cases, as that of Table XXXVIII,

fails to produce a water at all approaching in quality that obtained by merely shaking the original water with one of the solids and then filtering out the latter. Treatment of redistilled water with the solids generally shows a considerable improvement over the effects of simple redistillation. Sometimes it shows a comparatively slight improvement over the effects of the same treatment on the ordinary distilled water, and sometimes, as in Table XL, it fails to show as great a beneficial effect.

The manner of action of the solids in correcting the toxicity of ordinary distilled water will now be considered. The fact that practically the same effect is produced by four solids as different as are carbon black, ferric hydrate, aluminum hydrate, and quartz flour is at least a very strong indication that the benefit derived from the use of these is not due to their chemical nature. It is hardly reasonable to suppose that these four solids all carry into the water material which acts as a stimulant to increase growth, and it is still less reasonable to suppose that they all contribute nutrient materials to the water and that the better growth obtained in the treated waters is due to these. As has been remarked above, the quartz flour used in these experiments was not washed. While relatively it is only slightly soluble, it still shows a marked solubility when tested by the electrical resistance. But the other solids were very thoroughly washed, and the traces of soluble material which might have been carried into the water by them must be considered as exceedingly minute. The amount of soluble substances entering the water from the culture bottles themselves was undoubtedly greater than that resulting from the treatment of the water by these solids.

As the ferric hydrate was prepared from chemically pure ferric nitrate and ammonium hydrate, the only impurities at all likely to occur were the chemicals used in its preparation and the single soluble product of their reaction, ammonium nitrate. Several experiments were performed to compare the effect produced by treatment with this ferric hydrate with that produced by the addition of small amounts of ferric nitrate to the water. In no case have we been able to produce by means of the nitrate in ordinary distilled water any response in the growth of the plants at all comparable to that exhibited in water previously treated with ferric hydrate. Cultures were set up, using ferric hydrate, which was only partially washed, so that when it was shaken with the water and filtered out the filtrate showed a slight increase in conductivity over that of the water used. In the cultures with ferric nitrate a sufficient quantity of this salt was added to produce the same increase in conductivity as that brought about by treatment with the hydrate.

Calculating the transpiration in this experiment to a basis of 100 for the plants grown in ordinary distilled water, the increase produced by

treating this water with ferric hydrate was 328 per cent, while the plants grown in the ferric-nitrate culture gave a decrease of 7 per cent in transpiration.

It is perfectly clear from this result that ferric nitrate in the amount here used was not appreciably beneficial; on the other hand it appeared to be slightly injurious. Ferric nitrate was tried in other experiments at still lower concentrations, and while it sometimes showed a slight increase in growth this never amounted to anything at all comparable to that produced by treatment with the hydrate.

Still further evidence on this question of the possible action of soluble impurities in the ferric hydrate was obtained from the use of a sample of this solid prepared from ferric chlorid and sodium hydrate and washed in exactly the same way as was the hydrate commonly used. The result showed that this hydrate, which must be free from ammonium and nitrate ions, probably having slight amounts of sodium and chlorine ions in place of these, produced the same effect as did the hydrate prepared in the former way.

From the evidence presented in the preceding paragraphs it seems clear that the beneficial effect produced upon ordinary distilled water by treatment with carbon black and ferric hydrate can not be attributed even in a small degree to the presence in these solids of small amounts of soluble impurities. If, then, the effect is not produced by an addition of material to the water the only alternative is the conclusion that it must be due to the removal of some material therefrom. It seems fairly certain, therefore, that the treatment of the ordinary distilled water of these laboratories with insoluble solids, as described in the foregoing paragraphs, results in the removal from solution in the water of all or nearly all of the toxic substances which are originally present and which are markedly injurious to wheat seedlings. Since the washed solid keeps indefinitely, it can be used for preparing nontoxic water at the worker's convenience and according to his needs. It may even be taken to places where it is difficult or impracticable to operate a glass still, and the worker may enjoy the assurance that practically nontoxic water may be obtained at almost a moment's notice and at any time.

#### SUMMARY.

In the preceding pages it has been shown that the ordinary distilled water of these laboratories, like that of most other laboratories, is generally toxic to seedlings. This toxicity varies considerably from time to time, according to conditions which are difficult to determine and which are probably very complex, depending, no doubt, as is suggested by Lyon's work, in part upon the variations in the original water, and in part upon conditions of distillation and preser-

vation of the supply. It is certain that the water-conducting system, of block-tin pipes and brass cocks, used in these laboratories has a bad physiological influence on the quality of the water which has stood therein.

Simple redistillation in glass is not sufficient to correct the toxicity of the ordinary distilled water; at best it merely improves the quality of the water. Redistillation from a retort to which various substances, such as sulphuric acid and potassium dichromate, have been added gives much better water than simple redistillation, but even this treatment fails to correct the toxicity entirely. Redistilling from sulphuric acid and potassium dichromate and then from alkaline potassium permanganate, and condensing in platinum, produces water which is no better for physiological purposes than water redistilled from sulphuric acid and potassium dichromate and condensed in glass. Since no benefit was derived from the platinum condenser, its use is not to be advised.

The most satisfactory water which was obtained was prepared by shaking the ordinary distilled water with finely divided and thoroughly clean carbon black or precipitated ferric hydrate and then filtering out the solid. This and other lines of evidence show clearly that the improvement in the physiological quality of the water which is observed after treatment with the solids can not be ascribed to a possible addition of dissolved material. Evidence is presented that the finely divided solids when properly washed add no electrolytes, but actually remove material from the ordinary distilled water.

Even good redistilled water is generally improved by treatment with ferric hydrate or carbon black. This, together with the fact that mere redistillation does produce an improvement, seems to indicate that the toxic materials present in the ordinary distilled water are partly nonvolatile and thus removed by redistillation, and partly volatile with steam so as to reappear in the distillate. When sulphuric acid or potassium dichromate, or both these chemicals, are added to the retort, the main portion of the volatile injurious materials is held back or destroyed, but a certain degree of toxicity is usually still observed no matter how the water is redistilled. It appears that the absorbing solids remove both the volatile and nonvolatile toxic materials. Simple treatment of the ordinary distilled water with one of these solids appears adequate to remove practically all the injurious substances and to yield a water well suited for plant physiological purposes.

